IMPERIAL BUREAU OF PLANT GENETICS: HERBAGE PLANTS

BULLETIN No. 3.

THE BREEDING OF HERBAGE PLANTS: TECHNIQUE ADOPTED AT THE WELSH PLANT BREEDING STATION

Price 3/-

AGRICULTURAL BUILDINGS,
ABERYSTWYTH,
WALES.

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PREFATORY NOTE.

The information supplied in this bulletin has been brought together in response to the large number of requests that have been received for a detailed statement as to the methods employed in herbage breeding at the Welsh Plant Breeding Station, Aberystwyth.

No attempt is here made to review the methods that have been, or are at present, in use elsewhere, for the chief reason that all available information has been drawn upon in the evolution of the technique here described, while the matter will be dealt with more conveniently from a historical point of view elsewhere.

The underlying philosophy which has been responsible for the directions in which the work has been developed at Aberystwyth was the outcome of investigations undertaken prior to the foundation of the Station in 1919. Both Mr. Jenkin and I had been concerned with studies on the relation of sown to indigenous (= unsown) species in connection with seeds mixtures. The outstanding fact brought out by these and similar investigations was the marked difference from the strain point of view between the plants resulting from ordinary commercial seed of a number of the more important herbage species and those making a spontaneous or unsown (= indigenous) appearance on prepared swards.

It became obvious indeed that differences of a character of the same order as those so strongly emphasised by the late Professor Gilchrist as existing between wild white clover and the ordinary white Dutch clover of commerce were also to be met with amongst the grasses. It followed, therefore, when the foundation of the Station made it possible to undertake critical work, that at the outset we concentrated very largely upon an investigation of the types of the chief grass species met with on more or less natural habitats—very old pastures, hedges, thickets, roadsides, and the like.

The technique as now practised at Aberystwyth has been the outcome of some ten years of trial and error, and consequently considerable emphasis will be laid on the mistakes that have been made—for they have so very much to teach. It has only been possible to deal with the matter in sufficient detail—and it is absolutely essential to lay proper emphasis on detail—in a number of separate papers. A certain amount of over-lapping has been unavoidable and has indeed been necessary, since, for example, the methods adopted by Mr. Jenkin (the rye-grasses chiefly) and by myself (cocksfoot chiefly) are in certain rather important respects somewhat different.

My thanks, and those of this Bureau, are abundantly due to Mr. Jenkin and to Captain Williams for the trouble they have taken in preparing their contributions to this bulletin, and for the careful and accurate manner in which they have described the essential details of the methods they have evolved and now employ. Thanks are similarly due to Miss Rhoda Jones, B.A., of the staff of the Welsh Plant Breeding Station, for kindly seeing this bulletin through the press.

R. G. STAPLEDON.

AGRICULTURAL BUILDINGS,
ABERYSTWYTH,
June, 1931.

THE METHOD AND TECHNIQUE OF SELECTION. BREEDING AND STRAIN-BUILDING IN GRASSES.

BY

T. J. JENKIN, M.Sc.

INTRODUCTION.

The methods of procedure outlined in this paper assume the necessity of the closest possible study of individual plants with a view to the proper selection of those which will be subjected to breeding tests and finally of such as will be ultimately employed as parent plants.

The technique to be employed, therefore, begins with the collection of material in the field ; and with methods of handling and studying the plant material when it has been assembled at the research station.

COLLECTION OF PLANT MATERIAL.

As a standard of comparison plants derived from seed obtained from commercial sources have been grown and studied, but in the main the material chiefly employed has been derived from natural and semi-natural habitats. A few remarks as to the methods adopted relative to collection are, therefore, essential, and the more so since it is necessary to take certain precautions even in such a comparatively simple matter as this.

Collection of Seed.

From the point of view of obtaining general ideas as to the type of plants occupying any particular habitat it is sufficient to collect seed indiscriminately from a large number of plants without making any effort to keep the seed of each plant separate.

In the case of populations derived from such bulk collected seed in the event of a few plants showing very particular characteristics it is quite impossible to know whether such plants have been derived from one and the same mother plant or from different mother plants. It soon became apparent, therefore, that for critical comparisons with a view to subsequent selection and breeding it was necessary to collect seed from individual plants as such. On old swards as on many other types of natural habitats it is often difficult to ascertain definitely whether two inflorescences growing together represent a single plant or two separate plants. The unit for collection should, however, always be the single inflorescence, and it is therefore wisest to pluck only one inflorescence from amongst a group of inflorescences growing close together. If for any special reason it seems desirable to pluck more than one inflorescence from such a group each should be kept separate and be deemed to represent different plants. It may sometimes happen that an unexpected opportunity presents itself for collection when one may have no receptacles available for the reception of inflorescences. A good plan to adopt is to take one seed from each of a large number of fairly widely separated inflorescences and since these seeds necessarily represent different plants they may be brought home together in a match box or any other container the collector may have on his person. All seed employed with a view to single plant studies is sown in sterilised soil in boxes. Sowing is usually started in a heated greenhouse late in the autumn and continued all through the winter and into the early spring, and the seedlings are spaced out in the open in April, May, and June.

The actual procedure varies with the different species. The seeds of perennial ryegrass are large and can be easily separated. They are therefore spaced out in the boxes (64 seeds to a box of about 2 sq. ft.). Experience has shown this to be a satisfactory spacing, even if the seedlings have to remain in the boxes three or four months at a season of the year when growth

and development is relatively slow. In the case of species like the fine-leaved fescues, timothy, and the meadow grasses, which do not always germinate well, reserve boxes are also sown broadcast and the seedlings if required subsequently spaced out in boxes. The seed of cocksfoot is difficult to separate, and this species is always sown thinly broadcast and the seedlings subsequently pricked out into boxes. Italian rye-grass is always sown considerably later in the year, for if sown too early it comes to maturity in the seeding year.

Observations on Seedlings.

By spacing the seed in boxes it is possible to examine each seedling individually and the different seedling types produced can be noted, together with the proportions in which they occur. Most of the abnormal seedlings are in some way definitely defective, albinism and various types of chlorophyll deficiency being the most common defects. Such seedlings usually fail to survive and are themselves of no further importance.

Apart from these defective types, however, what may be termed " twin " seedlings have been observed to occur in several species. In these, two distinct plumules (or, more rarely, two " first leaves ") are produced by the same seed. In some cases these may be observed to be the result of very early branching, and in these cases the two plumules apparently represent a single embryo. In other cases it is impossible to decide without careful dissection whether two plumules are the result of extremely early branching or not. Such seedlings might be grown on and kept under observation, but even then it would be extremely difficult to decide unless the material were of such a kind that two individuals, if they occurred, might definitely be distinguished.

Two cases of " twin seedlings " which each consisted of two entirely independent embryos may be cited. In both cases the seed was examined after germination and re-planted.

(1) A seed produced in the course of work on viviparous sheep's fescue gave two plumules. This twin seedling was marked down and kept under observation until it reached maturity. Vegetatively the plant appeared to be a single entity, but two essentially different types of inflorescences were produced.

(2) A seed from a perennial rye-grass plant heterozygous for extreme dwarfism similarly gave two plumules, one of which developed into a normal plant, while the other became a typical dwarf.

The occurrence of such twins is of considerable importance in connection with breeding work. If the twin nature of a seedling be not detected the mature plant may give highly abnormal results when used as a parent. It may appear to be very highly self-fertile, while from self-pollination or from inter-crossing it may give peculiar seedling ratios. For this reason, twin seedlings of every kind are either discarded in the young stage or are marked down and kept under observation as such.

Collection of Plants.

A large amount of plant material has been collected from the old pastures of the Midlands and elsewhere. No effort was made to select type plants on the spot, but the aim was simply to obtain a large amount of representative material. The early spring was considered to be the best time for making such collections. A field was traversed in several directions and a large number of small sods about 3in. × 3in. dug up. All such sods as contained the species required were sent to Aberystwyth. On arrival the sods were broken down and the required species separated out. The rootings of the species so separated were carefully looked over and if necessary further broken down to individual tillers in order that there should be no possibility of an ultimate separation representing parts of two different plants, the aim again being to keep individual plants separate. The lesser of two evils undoubtedly is to have recorded as " separate " two rootings which in fact represented the same plant. In order to ensure that the same plant is not twice represented it is of course only necessary to take but a single organic entity from each sod dug up—a procedure which has much to recommend it.

The ultimate rootings having been thus obtained they were spaced out in boxes and subsequently transplanted to observation plots for detailed study.

Single Plant Beds.

Single plants, whether derived from seeds or rootings are usually set out in rows of 10 plants. The spacing varies for the species, but the rows are usually set 2 ft. apart. Every precaution is taken to keep the ground as clean as possible, and the Rototiller and the hand hoes are kept in constant use when conditions and the stage of growth of the plants permit. Most of the species under study are quite insensible to close hoeing and to cleaning operations in general. Perennial rye-grass is, however, particularly intolerant of close hoeing, and the plants have always to be " earthed up " after thorough cleaning. Plants of perennial rye-grass have a rather shallow rooting habit, and in the absence of trampling show a tendency to grow away from the soil.

Observations on the Plants in the Year of Planting.

As a rule but little critical data are obtained on the plants in the seeding year. It is, however, essential if it is desired to obtain such data that all lots to be directly compared should have been sown at the same time—for experience has shown that a relatively small difference in sowing date may make a very appreciable difference in development in that particular year. In practice the collections representing a particular species are always sown in convenient groups, and a few characteristic lots are included in each group to act as a standard of comparison. This safeguard has been found to be particularly useful where such characters as the development of inflorescences in the seeding year are to be compared.

During the seeding year it not infrequently happens that a certain amount of automatic or natural selection takes place. Thus, in the case of timothy, cocksfoot, and meadow foxtail, and especially in populations derived from foreign seed, quite a large number of the plants are likely to become so badly rusted that they are killed right out by the second year.*

PRELIMINARY SELECTION OF POTENTIAL PARENT PLANTS.

The serious study of single plant populations commences with the second year, and the preliminary selections are usually made at the close of the growing season. The single-plant blocks as a whole are not, however, broken up until the third year, since winter behaviour can be studied more accurately in the second than in the first winter.

Many thousands of single plants come for study each year, and the investigator has, therefore, to depend upon qualitative notes and upon his judgment, and consequently selection is made on the basis of appearance in relation to plant type and origin and the ultimate purpose in view.

With a view to future breeding work definite data on two well-defined and important characteristics can usually be obtained at this stage.

Growth Stage.

The first characteristic is the time at which a particular plant reaches a definite growth stage. As the result of experience an arbitrary standard has been taken to form the basis of critical comparison. This basis is the date (and the condition of the plant) when three inflorescences show through the mouth of the sheath—one or even two inflorescences may in rare cases appear precociously.

Observations are made on all the plants not less often than three times a week. The evidence from this method of comparison has proved of the greatest value in comparing different lots. It is even more valuable in connection with breeding work, as it would be very undesirable to match up for the establishment of a strain plants which differ widely with regard to the time at which they reach their various growth stages.

Male-Sterility.

In perennial rye-grass it has been found that male-sterile plants even though fully female-fertile are often somewhat more vigorous than normal plants. Such plants, however, as will be shown below, should only be selected and used for breeding for special purposes and, therefore, normally, no matter how attractive otherwise, these male-sterile plants should be rigidly rejected.

* The rust fungi responsible are : for timothy, *Puccinia Phlei-pratensis* ; for cocksfoot, *Puccinia glumarum*, and for meadow foxtail, *Puccinia perplexans*.

FINAL SELECTION OF POTENTIAL PARENT PLANTS.

The investigator will have had to rely on his judgment to have selected perhaps 100 plants from a population of quite 5,000. The next stage is to subject these select plants to more critical examination. The methods adopted have been as under.

Clone Method.

The single plants are broken up into a number of plantlets and each clone is spaced out separately.

Each clone can be studied independently, while the plant as a whole can be subjected to replication and if desired studied on different habitats. The replication also permits of the collection of quantitative data if so desired. This method has been largely employed especially with cocksfoot, and is still in use for certain species.

Tiller Beds.

The selected plant is broken up into tillers which are planted out 2 inches apart in a " broadcast " manner. By this method the single plant can be studied under conditions approximating to those of an ordinary sward. This method (which is also dealt with by Professor Stapledon in his article in this bulletin) has been employed chiefly with cocksfoot in connection with ecotypical selection when a number of phenotypically similar plants have been used for the formation of a single bed.

Tiller Rows.

The selected plant is again broken down to tillers or very small plantlets, and these are planted rather densely in single file in a tiller row. Under these conditions the identity of the single tiller is soon lost, and the row as a whole forms the unit of study. A vigorous plant of perennial rye-grass at the end of its second season will supply sufficient material to make two 5-ft. rows of this kind. The great merit then of the " tiller " as of the clone method is that duplication becomes possible even when material is limited to but a single plant.

The best time for breaking up and transplanting under Aberystwyth conditions has proved to be the end of August or early in September ; if tiller work is done later than this, sufficiently good establishment is not obtained before the advent of winter. Cocksfoot has, however, been broken into clones (not more than 4 per plant) and successfully transplanted on this basis right through the winter, except during actual periods of frost.

Experience has shown that the tiller row shows up the individuality of the plant to a considerably higher degree than does the clone, while two plants ranged side by side in tiller rows are more easily and more accurately compared.

It has not been found satisfactory to attempt to obtain quantitative data on the tiller rows, since on the one hand only duplication is possible and on the other the cutting of the herbage would interfere with the collection of valuable observational information.

By the methods outlined it will have been necessary to have reduced the potential parents to a number sufficiently small to be subjected to breeding tests. Obviously all that will have been possible to do so far is to have selected plants as individuals—as types, and nothing as yet is known concerning their breeding characteristics.

ISOLATION AND SELF-POLLINATION.

Whatever breeding tests are undertaken, the first essential is to employ satisfactory methods for the isolation of one's plants, to develop a proper technique for selfing, and to arrive at some satisfactory standard for estimating and comparing self-fertility. At this stage it will be convenient, therefore, to refer briefly to the whole question of isolation as applied to single plants and to groups of plants. The following methods of isolation have been adopted at Aberystwyth : (1) by distance in the open ; (2) in greenhouses ; (3) by resort to specially constructed cages ; (4) by bagging in the greenhouse, and (5) by bagging in the open.

The methods employed for isolating by distance in the open, in greenhouses, and in cages are fully discussed by Professor Stapledon in his article (see p. 35), and consequently need not be considered here. The technique adopted relative to single plants (bagging) must, however, be considered in some detail.

Bagging in Greenhouses.

All the more critical work is conducted in cool greenhouses, and it has been found that bags made of thin glazed semi-transparent paper are the most satisfactory for the purpose. It is essential that the gumming should be well done, and it has been necessary more than once to reject a consignment of bags on account of faulty gumming. It is seldom that the corners are sufficiently well gummed, and it is part of the regular routine when bagging to fold back the corners and make them secure with ordinary paper clips in a dog-ear fashion.

The number of inflorescences enclosed will vary with the species and the size of inflorescences and of the bags. As few as 4—6 large panicles of cocksfoot may be used and as many as a dozen spikes of rye-grass.

Three chief precautions must be taken when bagging : firstly, to enclose the inflorescences soon enough so that there shall be no risk of stray pollen remaining viable within a bag long enough to effect cross-fertilization when the stigmas are exserted. Secondly, to close the mouth of the bag properly. To facilitate packing the upper stem leaves are removed from the panicles ; this also lessens the risk of including green fly and makes it easier to change the position of the bag, which is periodically necessary as a result of the elongation of the flowering stems. To close the mouth of the bag properly a strip of good cotton wool about 3 inches wide and 15 inches long is taken and into this the panicle stems, a little below the inflorescences, are individually rolled in such a way that no two lie side by side. An overlap of the end of the cotton wool strip at the finish forms a buffer between the tie, which is required to keep the stems in position, and the stems. This tie is not made very tight, but another tie at practically the same point is required to secure the mouth of the bag over the cotton wool pad. This outer tie can be made quite firmly without danger of injury to the stems if sufficient cotton wool has been used. Thus the container at this end is made absolutely pollen-proof. Thirdly, it is necessary to keep the bag rigid. For this purpose a bamboo stake is used, and the bag is attached to it at two points. At the mouth-end of the bag the tie is made over the cotton wool pad, at the other end it is attached to one of the clips used to keep the corners of the bag turned (Plate I, fig. 2.).

Bast (raffia) is used for all the ties.

Bagging in the Open.

The weather conditions at Aberystwyth—where rain, accompanied by high winds, is of frequent occurrence in the summer—render the matter of selfing plants on a large scale out of doors a very difficult and a very perplexing one.

Paper Containers.

The desirability of employing if possible absolutely pollen-proof material was at once recognised, and this led to experimenting with various types of treated paper or parchment. The glazed paper bags as used in the greenhouses proved to be quite useless for outdoor work, and ordinary parchment paper bags were equally unreliable. Paper cylinders steeped in melted wax were found to be both pollen- and weather-proof. Owing to poor ventilation the seed setting, however, was not good enough to justify the labour involved in preparing these containers.

Waxed paper sheets (a commercial commodity) were also tested, but since no method of gumming these was known the bags had to be hand-made by folding over the margins and pinning down with paper clips. These containers gave fairly satisfactory results, but the preparation of the bags was altogether too laborious.

In 1928 and 1929 trials were conducted with bags made of " vegetable parchment". This material proved to be both rain- and pollen-proof, but not sufficiently storm-proof when used in ordinary bag form, for no method of tying and supporting could be devised, which prevented the bags from chafing against the stake, and further than this, no device had been provided for keeping the bag open and rigid, and the sides of the bag from sagging in on the panicles.

In 1930 the vegetable parchment was tested in a new type of bag which took the form of a sleeve (the " topless and bottomless " bag of the trade).

The sleeve was fitted over a wire spiral, the loose ends of which were turned well in so that they did not come in contact with the bag. The sleeve *cum* spiral was employed in a manner similar to that adopted with the fabric " balloons " (described below). This material used in the manner described promises to be very satisfactory—and there is reason to believe that it affords the solution to the problem of absolute isolation compatible with reasonable conditions for seed setting for plants which it is desirable to self in the open.

Cotton Fabrics.

Covers made of various cotton fabrics have been continuously and extensively used at the Station. Before discussing questions connected with the fabric as such it will be convenient to describe the methods of caging (for single plants) that have been adopted.

The appropriate fabric is now purchased in circular (seamless) pillow-case form.* The circular fabric is cut into lengths of about 3 to 4 feet, the diameter being about 15 inches. The sleeves so made are stretched over an appropriate frame. The frames now employed are made of two hoops of hazel kept apart by six struts of the same material. Care has to be taken to make the frames perfectly smooth on the outside so as to avoid any risk of tearing the material.

The sleeve stretched over its frame constitutes the balloon. The process of bagging is relatively simple and can be conducted rapidly. Stout dahlia stakes are driven down hard against the plants to be caged. A good wad of cotton wool is tied round the stake near the top and another at the position where the panicles will be tied to the stake. The selected panicles† are wound into a separate wad of cotton wool and loosely tied to the stake. The balloon is then brought over the top of the stake and the panicles, the fabric at the upper end of the balloon is drawn together and tied firmly round the cotton wool wad at the top of the stake and the fabric at the lower end of the balloon also tied firmly round the cotton wool pad supporting the panicles. The cotton wool pad around the panicles must be sufficient to permit of considerable pressure (Plate I, fig. 3). It is essential to use string since the ties must be strong in order to keep the balloon properly stretched.

At harvest time the stems of the panicles are cut below the balloon and the whole structure (stake with balloon) removed bodily. The balloons are generally kept standing in a greenhouse until the seed is rubbed out.

The Adequacy of Cotton Fabric as a Protection against Stray Pollen.

The cotton fabric has three great advantages : it permits of the passage of air into the cage, it is remarkably storm-proof, and used as balloons allows of rapid caging. It has, however, to be recognised as exceedingly doubtful whether the meshes of any ordinary cotton fabric are smaller than the size of a grass pollen grain. In a densely woven fabric the loose ends of the individual fibres, however, go a long way towards filling up the actual mesh. Obviously, it would be unsafe to assume that even the most carefully selected fabric was absolutely pollen-proof unless it had been possible to subject the material to critical test. From a practical point of view, it is just as important to know the extent to which a good fabric may afford protection against stray pollen as whether it affords absolute protection. For in many aspects of practical plant breeding a distinction has to be made between what is effective for the purpose in view and what is absolute.

It was not until 1928 that suitable material was available at the Station for the purpose of conducting critical experiments to test the adequacy of different cotton fabrics.

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* This does away with the necessity of a seam. The needle holes become dangerously large in relation to the size of the mesh of the fabric and to the size of the pollen grains when a sewn sleeve is stretched over a frame.

† It is even more important to remove the leaves from the panicle stems in connection with outdoor bagging than in the greenhouse.

EXPERIMENT B. 183.

This experiment was designed to test three types of cotton fabric, and was divided into four sections as under :—

A. Open pollinated plants.

B. Plants protected by a fabric of the cheese cloth type with very open texture.

C. Plants protected by a medium fabric (lawn) of fine texture ; fabric indeed of such a character that it might easily be used for self-pollination work unless closely examined.

D. Plants protected by a very dense and rather heavy fabric which represented the " standard " used at the Station in recent years. This fabric is the most closely woven that it has been found possible to procure.

These descriptions are, of course, inadequate to convey an accurate idea of the actual types of fabrics used, but through the kind assistance of the Shirley Institute, Didsbury, Manchester (Director of Research : Dr. R. H. Pickard, F.R.S.)* photographs (magnified) of each of the three fabrics have been obtained and are reproduced in Plate III. Further, the institute referred to kindly supplied the following report on the three fabrics :—

" The following are what we should consider reasonable specifications of these three bleached cloths :—

	Threads per inch.		*Count.*	
	Warp.	*Weft.*	*Warp.*	*Weft.*
No. 1 (D) ..	68	65	2/32's	16's
No. 2 (C) ..	100	80	55's	60's
No. 3 (B) ..	55	55	34's	48's "

The photographs show at a glance that the three fabrics differ greatly, and that fabric " D " is a particularly closely woven type. The specifications given above should make it possible also for other workers to procure fabrics of exactly the same type if they so desire. Small portions of the bags actually used in this experiment can be obtained from the Director of the Station on application.

A word may here be added concerning the selection of fabrics. The examination is made by means of a binocular microscope with a magnification of about fifteen, but it is necessary first of all to see that every trace of " filling " or " dressing " is removed. This is usually done by hard rubbing.

The Plant Material used.

The experiment was started in 1928, and was repeated in 1929 and 1930. In 1928 and 1930 non-red perennial rye-grass plants of Line 169 were alone used, and in each case they were scattered amongst a heterogeneous population of the same species derived from many different sources, but not including plants related to those used in the experiment. These non-red plants are homozygous recessives for anthocyanin coloration, while the plants of a normal population are of the red-base type,† and naturally occurring heterozygous plants are exceedingly rare. The extent to which stray pollen can penetrate the balloon fabric may be ascertained by examination of the resulting seedlings. All seedlings which develop anthocyanin coloration must of necessity be the result of inter-crossing ; non-red seedlings may or may not be the result of self-pollination.

Plants of this line are also usually of low self-fertility, so that some idea of the amount of pollen which has effectively penetrated the fabric cover may be obtained by a comparison of the relative amount of seed set, using in this case the standard of number of seeds per hundred spikelets enclosed.

Owing to circumstances connected with the breeding work programme somewhat different plant material had to be used in 1929. Some non-red plants of Line 169 were again available,

* The research institute of the British Cotton Industry Research Association.

† No exception to this rule has been met with at Aberystwyth, and only one " natural " plant heterozygous for the factor concerned has been identified.

but they had been produced by a different method of breeding and were, on the whole, more highly self-fertile. Moreover, they were situated in a less favourable position, since a proportion of the surrounding population consisted of related plants—some of them heterozygous for base-colour. The number of non-red plants available in this season was, however, insufficient for the purpose of the experiment, and the deficiency was made up by using male-sterile plants. Of necessity, such plants cannot produce seed from self-pollination and they are, therefore, quite as suitable for an experiment of this kind as the non-red plants. In the case of this type of plant it is necessary only to compare the amount of seed set.

Results.

The results obtained are given in detail in Tables I—IV, so that if desired the individual results may be directly compared. It will be noticed that those for 1928 differ from those for 1929 and 1930 in that one and the same plant was not used for open pollination and for isolation. This fact, however, does not in any way seriously affect the comparisons to be made.

Fabric B.—" cheese cloth."

A glance at the different tables will show that the results for this type of covering do not differ in any essential from those obtained by open pollination. There is, in some instances, a suggestion that there has been some interference with seed setting, but in this respect the results are not consistent. With the male-sterile plants, seed setting with " cheese cloth " was actually higher on the basis of seed per 100 spikelets than from open pollination. In the proportions of the different seedling types there is certainly no significant difference. It is, therefore, obvious that as a protection against stray pollen this material is useless.

TABLE I.—*Test of cotton fabrics. Part* I. 1928.

Plant.	Covering.	Spike-lets enclosed	Heavy seeds.	Heavy seeds per 100 spkts.	Seed sown.	Seeds ger-mi-nated.	Percent. ger-mina-tion.	Seedlings.		
								Red base.	Non-red.	Indef-inite.
295(1)4	"A" none	191	390	204.2	388	201	51.8	**197***	0	4
295(1)6	,, ...	204	427	209.3	400	343	87.8	**331**	2	10
295(1)7	,, ...	268	267	99.6	267	103	38.6	**97**	1	5
296(1)4	,, ...	183	354	193.4	354	204	57.6	**192**	1	11
								817	4	30
295(1)1	" B " cheese cloth ..	279	330	118.3	330	191	57.9	**187**	2	2
295(1)8	,, ..	298	621	208.4	500	446	89.2	**429**	6	11
296(1)7	,, ..	151	117	77.5	117	113	96.7	**109**	3	1
296(1)8	,, ..	294	126	46.2	126	109	86.5	**105**	1	3
								830	12	17
295(1)5	"C" medium fabric	248	151	60.9	150	138	92.0	**105**	28	5
295(1)9	,, ..	191	55	28.3	55	52	94.5	**50**	0	2
296(1)3	,, ..	145	59	40.7	59	53	89.8	**50**	1	2
								205	29	9
295(1)2	"D" standard fabric	448	10	2.2	10	9	90.0	**1**	7	1
295(1)3	,, ..	166	0	0	0	0	0	0	0	0
295(1)11	,, ..	150	5	3.3	5	5	100.0	**1**	3	1
296(1)6	,, ..	177	0	0	0	0	0	0	0	0
								2	10	2

* Figures in heavy type indicate seedlings which could not have been produced by self-pollination.

TABLE II.—*Test of cotton fabrics. Part* II, 1929. *Section (a) : Non-red plants. Seedlings represented in percentages.*

Plant.	Covering.	Seeds per 100 spikelets.	Percent. germination.	Seedlings.				
				Green.			Percent. chlorophyll deficient and lethal.	Total seedlings (actual).
				Percentage				
				Red-base.	Non-red.	Indefinite.		
270(2)21/6	None (A) ..	87.4	97.0	**67**†	24	2	7	97
	Fabric B ..	30.1	95.9*					
271(2)6/8	None (A) ..	202.4	73.8	**42**	26	19	13	124
	Fabric B ..	136.4	79.5	**43**	28	23	6	159
270(2)22/5	None (A) ..	177.1	79.8	**65**	9	25	1	99
	Fabric C ..	21.5	84.4	**89**	11	0	0	27
271(2) 6/15	None (A) ..	165.3	86.3	**58**	10	16	16	107
	Fabric C ..	65.3	82.6	**35**	24	19	23	89
271(2) 6/4	None (A) ..	107.0	82.5	**39**	27	8	26	165
	Fabric D ..	63.6	59.7	0	54	7	39	74
271(2) 6/9	None (A) ..	170.1	87.8	**45**	20	16	19	130
	Fabric D ..	36.6	67.6	0	40	23	37	48

* Seedlings accidentally discarded without full examination.
† Figures in heavy type indicate seedlings which could not have been produced by self-pollination.

TABLE III.—*Test of cotton fabrics. Part* II, 1929. *Section (b) : Male-sterile plants, possibly heterozygous for chlorophyll deficiency.*

Plant.	Covering.	Spikelets enclosed	Heavy seeds.	Heavy seeds per 100 spkts.	Seeds sown.	Seeds germinated.	Per cent. germination.	Seedlings.		
								Green	Chl. def.*	Indefinite.
413(1)8	A. None	91	275	302.2	100	100	100.0	94	6	—
	B. Cheese cloth ..	182	849	466.5	100	99	99.0	94	5	—
415(1)5	A. None	95	392	412.6	100	96	96.0	96	—	—
	B. Cheese cloth ..	211	1042	493.8	100	92	92.0	92	—	—
413(1)13	A. None	81	318	380.2	100	88	88.0	88	—	—
	C. Medium fabric ..	158	205	129.8	100	94	94.0	94	—	—
415(1)7	A. None	104	314	301.9	100	94	94.0	88	6	—
	C. Medium fabric ..	209	282	134.9	100	92	94.0	92	2	—
413(1)15	A. None	84	99	117.9	99	93	93.9	91	2	—
	D. Standard fabric ..	182	0	0.0	0	0	0.0	—	—	—
415(1)10	A. None	84	238	283.3	100	98	98.0	95	3	—
	D. Standard fabric ..	162	0	0.0	0	0	0.0	—	—	—

* Chlorophyll deficient.

TABLE IV.—*Test of cotton fabrics. Part* III. 1930.

Plant.	Covering.	Spikelets enclosed	Heavy seeds.	Heavy seeds per 100 spkts.	Seeds sown.	Seeds germinated.	Germination per cent.	Seedlings. Red-base.	Seedlings. Non-red.	Seedlings. Indefinite.
461(1)1	None (A)	185	439	231.9	300	224	74.7	**215**†	9	0
	Fabric B.	216	417	193.1	300	260	86.7	**249**	10	1
446(1)1	None (A)	169	150	88.8	150	127	84.6	**119**	7	1
	Fabric B.	192	174	90.6	174	121	69.5	**115**	4	2
433(1)3	None (A)	166	106	63.9	300	279	93.0	**271**	1	1
	Fabric B	178*	25	17.6	25	17	68.0	**17**	0	0
459(1)1	None (A)	199	502	252.3	300	274	91.3	**264**	4	6
	Fabric B	218	531	243.6	300	276	92.0	**272**	1	3
483(1)1	None (A)	121	407	336.4	300	289	96.3	**281**	7	1
	Fabric C	322	589	182.9	300	275	91.7	**263**	11	1
499(1)2	None (A)	209	697	309.5	300	258	86.0	**252**	6	0
	Fabric C	284	399	140.5	300	283	94.3	**268**	15	0
433(1)2	None (A)	191	250	130.9	249	172	69.1	**166**	0	6
	Fabric C	238	102	42.9	102	85	83.3	**83**	2	0
433(1)5	None (A)	167	508	304.2	300	266	88.7	**261**	4	1
	Fabric C	208	321	154.3	300	279	93.0	**271**	1	1
499(1)1	None (A)	216	729	337.5	300	280	93.3	**274**	6	0
	Fabric D	235	9	3.8	9	7	77.8	**2**	3	2
270(2)22/1	None (A)	334	620	185.3	300	256	85.3	**254**	0	2
	Fabric D	170	1	0.6	1	1	100.0	**1**	0	0
433(1)1	None (A)	215	349	160.2	300	269	89.3	**262**	6	1
	Fabric D	206	0	0.0	0	—	—	—	—	—
460(1)1	None (A)	247	756	306.1	300	261	87.0	**255**	5	1
	Fabric D	266	0	0.0	0	—	—	—	—	—

* Inflorescences very badly bent in cage.

† Figures in heavy type indicate seedlings which could not have been produced by self-pollination.

Fabric C—medium fabric.

There is no doubt that this fabric has definitely affected seed setting, while in several instances a small but probably significant change in the proportions of the seedling types is also shown. At the same time the figures indicate again quite definitely that the material provides totally inadequate protection against stray pollen, since on the figures for 1928 and 1930, 94 per cent. of the seed set was due to pollen penetration.

Fabric D—standard fabric.

The results for this fabric show a great decrease in seed setting, but those given in Table II appear at the same time to show a much smaller decrease than the others. This is undoubtedly due to the fact that, as already pointed out, these plants were more highly self-fertile.

With this fabric, the male-sterile plants in 1929 failed to set a single seed, and the non-red plants in the same year produced no red-base seedlings. These results, therefore, suggest that the fabric is pollen-proof, but the seed produced in 1928 and 1930 gave a total of five red-base seedlings—nearly 23 per cent. of the total seedlings obtained. On the other hand, these five

seeds only represented one in about 760 possible seeds, calculated on the basis of the results obtained from open pollination. The former figure represents a result obtainable with plants bordering upon absolute self-sterility ; the latter, that obtainable with self-fertility equal to open-pollination. In practice, therefore, the reliability of the seed produced under this type of cover depends almost entirely upon the degree of self-fertility of the plant concerned. Where a plant has produced only very few seeds, the results obtainable are of little value, since there can be no guarantee that the seed has resulted from selfing, but where a plant has set an abundance of seed, they can be depended upon except for critical work.*

General Considerations.

These results are of the highest importance in connection with methods of isolation under outdoor conditions. With all three fabrics used, the " balloon " was perfectly made, so that no crevice was left through which stray pollen might enter. Any pollen that entered the container after bagging, therefore, must have done so through the fabric itself. Fabric " C " (medium texture) might have been expected to be at least highly effective, but the results are extremely disappointing. It is, therefore, evident that even for " practical " as contrasted with " critical " work, great care is necessary both in the selection of the fabric and with regard to the structure of the container, and no latitude can be allowed which can possibly be avoided. From the practical point of view the type of container used in this experiment, and the standard fabric " D " have been proved to be highly effective even though the fabric may not be absolutely pollen-proof.

METHODS OF PRESENTING SELF-FERTILITY RESULTS.

Seeds per Inflorescence.

This method fails to take into account both differences in number of spikelets per inflorescence and number of florets per spikelet. It should, therefore, only be used where these particulars cannot be ascertained except with the greatest difficulty, and where no other simple method is applicable. The outstanding instance where its use is inevitable except on a very limited scale is that of cocksfoot. In this species, the spikelets are so densely packed in the clusters that on any large scale the amount of labour involved in the separation of the spikelets is prohibitive.

Seeds per Hundred Spikelets.

This is the method most extensively used for species bearing multi-flowered spikelets, and practically all the results in the present paper are given in terms of this standard. It takes into consideration the relative sizes of the inflorescences and, on the whole, makes a fairly satisfactory standard for such species as the rye-grasses and the fescues. It does not, however, take into account differences in the number of florets per spikelet.

Seeds per Thousand Florets.

Where a very large number of results are to be obtained it is quite impossible to ascertain the actual number of florets involved except where absolute accuracy is essential. For practical purposes, results for species bearing multi-flowered spikelets have, therefore, been recorded on the basis of the estimated number of florets. The number of spikelets per inflorescence is first of all ascertained and a particular spikelet situated in a particular position in the inflorescence is taken as representative of that inflorescence. Thus the estimated number of florets per inflorescence can easily be determined. For the rye-grasses, the " standard spikelet " is the middle spikelet in an inflorescence bearing an odd number of spikelets and the uppermost spikelet in the lower half of an inflorescence of even spikelet number. This has been found to

* This discussion is based upon the results as they stand. It may be mentioned, however, that owing to the fact that the plants were not very robust either in 1928 or 1930, isolation was delayed until the last possible moment in order that the flowering stems might elongate. It is conceivable that these five seeds were in fact the result of pre-pollination, and not due to pollen penetration.

give a result slightly above the actual, but since the standard is uniform for all inflorescences and units the error so introduced is not of serious importance. The greatest difficulty involved (and this applies equally to actual counts of all florets) is the fact that at the tip of the spikelet the floret may be fairly well developed or only rudimentary, and whether the uppermost floret should be included or not must be left to the judgment of the individual unless it happens that it has produced a caryopsis.

In the fescues, the standard spikelet is the lowest of those borne singly towards the tip of the inflorescence.

This method should probably always be used for the rye-grasses in cases where actual floret counts are impossible, since in these grasses the number of florets per spikelet may vary from less than five to more than ten even in the same plant if grown under widely different conditions.*

Seeds per Hundred Centimetres.

This method is used in such species as timothy and meadow foxtail, unless accuracy in the result is of such importance as to justify spikelet counts. All the inflorescences in a unit are measured, the total length ascertained, and this used as the basis. It cannot give a perfect comparison between plants of widely different types since it does not allow for density of inflorescence and other small differences in detail.

The Effect of Method of Isolation upon Self-fertility Results.

Extensive *ad hoc* experiments on this point have not been carried out, but a great deal of information has been incidentally obtained. The most extensive data available were obtained under greenhouse conditions and afford a comparison between units which have been isolated for self-pollination only and those which have been used for the production of pollen in hand hybridization work. The methods used for the isolation of self-pollination units have already been described, and the particulars apply equally to " male " units, with the exception that the latter, of necessity, have to be opened for the extraction of pollen. This difference in treatment means that when pollen is to be extracted the inflorescences are much more vigorously shaken up than is ever the case with the self-pollination units, and it might, therefore, be expected that self-pollination is thus rendered more uniform. Moreover, the inflorescences are withdrawn from the bag when pollen is extracted,† so that the atmosphere in the bag is changed, and, presumably, made more nearly normal.

The difference in the treatment of the two types of unit is not very great, but the more vigorous shaking and the periodical renewing of the air in the bags in the case of the " male " units might possibly be expected to have some effect upon seed setting.

Variation in a Plant of low Self-fertility.

Perennial rye-grass plant 43 has consistently been found to be of low self-fertility, although it produces an abundance of pollen. Fourteen units of this plant have over a period of three years been isolated for self-pollination in the greenhouse. These units were only interfered with to the extent that the bags might have occasionally been lightly shaken up during flowering. On the average, each unit consisted of about five inflorescences, or 86 spikelets, giving a total of 1,200 spikelets enclosed. These units gave an average of 1.5 seeds per 100 spikelets. Six " male " units were similarly used in three seasons. The average unit size was now considerably larger, but it was by no means excessively large, since the average number of inflorescences per unit was about 15, and the average number of spikelets per unit about 286. The total number of spikelets in the six units was 1,717, and these produced an average of 1.1 seeds per 100 spikelets, so that the difference in the results obtained by the two methods, if at all significant, is in favour of the " self-pollination " (rather than the " male ") method, using relatively small units.

* Some results are given in Table V (p. 17) in terms of seeds per 100 spikelets, and on the basis of seeds per 1,000 florets. These results illustrate the effect of the two methods of presentation.

† Accidental pollination of the florets of a male unit during the few moments they are exposed is extremely rare if ever it occurs, and, therefore, this factor cannot have any appreciable effect upon the seed setting result.

Variation in a Plant of relatively high Self-fertility.

Perennial rye-grass plant 48 is much more highly self-fertile than plant 43, and the results obtained are therefore of greater interest. The methods used were again mainly those used with plant 43 (methods " B " and " C " of Table V), but two results have also been obtained from the use of fabric balloons.

TABLE V.—*Results obtained from plant 48 self-pollinated in different seasons and under different conditions.*

Unit number.	Year.	Method.*	Spikelets enclosed.	Times opened.	Seeds.	Seeds per 100 spkts.	Seeds per 1000 flts.	Av. florets per spikelet.
17	1922	B	357	—	303	84.9		
18	1922	B	343	—	355	103.5		
	1924	B	496	—	709	142.9		
18	1925	A	486	—	913	187.9		
1	1926	A	861	—	824	95.7		
149	1926	B	749	—	1248	166.6		
150	1926	C	613	4	1141	186.1		
88	1927	B	322	—	227	70.5		
110	1927	B	338	—	476	140.8		
126	1927	B	307	—	301	98.0		
109	1928	B	252	—	148	58.7	124.7	4.7
111	1928	B	181	—	216	119.3	156.3	6.4
112	1928	B	290	—	219	75.5	131.3	5.7
130	1928	B	173	—	84	48.6	105.5	4.6
135	1928	B	227	—	271	119.4	217.0	5.5
110	1928	C	466	3	386	82.8	203.1	4.1
131	1928	C	351	3	655	186.6	365.1	5.1
170	1929	B	147	—	122	83.0	193.0	4.3
181	1929	B	151	—	88	53.0	129.2	4.1
119	1930	B	154	—	291	189.0	224.9	8.4
125	1930	B	179	—	122	68.2	111.8	6.1
162	1930	B	174	—	178	102.3	148.2	6.9
163	1930	B	161	—	107	66.5	93.7	7.0
157	1930	C	163	1	105	64.4	111.1	5.8
158	1930	C	195	1	88	45.1	63.9	7.1
244	1930	C	158	3	41	26.0	61.6	4.2

* Methods: A=Fabric balloon in the open.
B=Usual method of self-pollination in greenhouse.
C=Used for the extraction of pollen in greenhouse.

The outstanding feature of the results taken as a whole (Table V) is actually not the variation due to method, season, or size of unit, but the wide range of variation in the same plant irrespective of these factors. Certain individual results might certainly be picked out which would appear to indicate a direct effect of one of these factors. For instance, the results for 1926, if considered alone, might be taken to indicate a difference due either to method or size of unit or a combination of both factors, but it will be found that in other seasons differences quite as great have been obtained by a single method, or with units of approximately the same size, or with the conditions of 1926 reversed. Thus in 1930, units 119 and 163 were very nearly of the same size, and the same method was used. Yet the results differ even more widely than the extremes of 1926. Similarly units 119 and 244 of the same year (1930), again approximately of the same size but using different methods, gave results which are the reverse of those of 1926.

The method of presenting the results appears to be of some importance in this case. When presented on the basis of seeds per 1,000 florets, as in the case of the results for 1928, 1929 and 1930 (Table V), there is evident a certain degree of "levelling up". There is also a suggestion that if a sufficiently large number of results were available a definite correlation might be found between the number of florets per spikelet and the number of seeds set per 1,000 florets, particularly in the same season. The number of florets per spikelet is again a symptom of the relative vigour of the plantlet. The more vigorous the plant, the greater the number of florets per spikelet. There is, therefore, a possibility that the vigour of the plant may have a considerable effect upon the amount of seed set from self-pollination, but there appear to be still other and more subtle factors which affect the result.

That the wide variation found in the case of the plant under discussion is not due to imperfect isolation is proved by the fact that the genetical seedling results obtained are not appreciably affected by the differences in seed setting. The genetical constitution of plant 48 in respect of several characters is known, and any serious contamination from foreign pollen would at once be detected in the proportions of the various seedling types produced.

It is, therefore, evident from these results that where a number of methods are fairly satisfactory a few individual results are insufficient to determine whether any particular method is definitely superior to another, unless all other factors are also taken into consideration. These results show further that it is no easy matter to compare the self-fertility of different plants, although it is evident that overlapping between such types as plants 43 and 48, where the difference in capacity for seed setting is great, is not likely to occur. Moreover, in the case of plants which are moderately vigorous a few random tests, on the laws of chance, are more likely to give results somewhere near the average than results at either extreme. Thus, the first two results for plant 48, obtained in 1922, show a self-fertility of 84.9 and 103.5 seeds per 100 spikelets respectively, as compared with an average of about 116 seeds per 100 spikelets for 26 individual results obtained over eight different seasons.

A further point of interest brought out by these results is the fact that so long as vigour can be maintained no definite change in self-fertility is brought about by advancing age. This is equally true for plant 43 and for plant 48—the former of low self-fertility and the latter relatively highly self-fertile.

INTER-CROSSING.

Two main methods together with modifications of these may be used for inter-crossing. The first (hand hybridization) involves the emasculation of the florets with subsequent pollination, while the second (mutual pollination) consists merely of enclosing the inflorescences together for mutual pollination. These two methods differ greatly both in the amount of labour involved and in the type of result to be expected.

Hand Hybridization.

Experimental work on the technique of hand hybridization was undertaken in 1921 for the purpose of developing a technique suitable for herbage grasses. This experimental work was successful, and a bulletin was issued in which the main method was described (Jenkin, 1924), certain results given, and some aspects of the problem discussed. This publication is now out of print, and for this reason methods of hand hybridization may again be described briefly.

Treatment of Parent Plants.

In the artificial hybridization of wind-pollinated species, perfect control of pollen is of first importance. This is impossible under outdoor conditions, and therefore plants to be used for this purpose in any particular season are planted in pots in the preceding autumn. A plantlet should not be too large in relation to the size of the pot, otherwise it will produce numerous inflorescences with weak spikelets and florets rather than fewer inflorescences with bolder spikelets and florets.

The Greenhouse.

Some time in advance of flowering the plants are taken into a cool greenhouse and there they remain until the seed has been harvested. They stand on the ground and therefore in order that they may get as much light as possible, the brick foundation of the house should be no higher than the top of the pots. The house should also be capable of thorough ventilation during the day-time, so that the plants may remain healthy and flourishing, while on the other hand it should be so constructed that when closed down it will be practically draught-proof.

The Male Unit.

A plant which is to be used for crossing is also self-pollinated by the method already described. At the same time another slightly larger unit is isolated in the same way for the provision of pollen.

The Female Unit.

The female unit usually consists of four, five, or six inflorescences. Since emasculation must be done a few days in advance of flowering it is unnecessary to isolate in advance of emasculation.

Emasculation.

The principal method may be described in some detail for *Lolium perenne*. The inflorescences to be used are " matched up " as well as possible in order that they may all flower within a relatively short range of days. A few of the upper spikelets of the inflorescence are discarded, and emasculation proceeds from the uppermost of those left towards the base in order to avoid handling of florets already emasculated. Usually, all the florets of a spikelet except the two lower ones are again discarded, and the upper of those left emasculated first. In order to avoid piercing the anthers a pair of forceps which are relatively broad and flat-pointed and blunt are used for emasculation in preference to those which are sharp or needle-pointed. When all the inflorescences have been dealt with, they are bagged up in exactly the same way as units which are to be self-pollinated.

The method for other species will vary somewhat from this scheme, but it is essentially similar for all species with multi-flowered spikelets. In *Phleum*, *Alopecurus*, and *Phalaris*, the glumes are strongly keeled and the florets relatively small. The spikelets in these are also very crowded, so that very severe thinning is necessary. Moreover, it is advisable to deal in detail with a relatively short length of spike, and to cut away the greater part rather than to leave a few scattered spikelets over a greater length, since after severe thinning, the rachis becomes dried up. For successful work with *Phleum* it has been found advisable to use more finely pointed forceps with the aid of a lens,* and this would probably be useful also in dealing with *Alopecurus* and *Phalaris* as well as with other small-flowered species.

Pollination.

The house is usually closed down about an hour before pollination begins in order that any floating pollen which has entered from outside may have time to settle down. During this time also the atmosphere becomes relatively humid—a condition which is destructive to pollen directly exposed to it.

Each day as long an interval as possible is allowed between the time a species comes into flower and the time when pollination is begun in that species, since the chances of accidental cross-pollination are thus minimised. To meet all cases and also as a matter of convenience, the house is usually closed down (except roof vents) about 6 p.m. B.S.T., and closed down absolutely and made as draught-proof as possible about 7 p.m., when the work of pollination for the day begins. The pollen of two different plants of the same species is never used at the same time within the same house. A male-unit bag is vigorously shaken and the pollen is assembled in the

* That originally used was a small × 8 lens, but at my suggestion Mr. B. L. Sethi used a large reading glass in 1930.

creases towards its mouth. When the bag is gently removed no pollen is lost, and it can be run out on to a square of dark glossy paper which has been previously folded into a boat-shape and cut into a sharp point at one end. The male-unit is immediately re-bagged and the female-unit bag opened. The pollen is then lightly brushed on to each exserted stigma independently by means of a very small and soft brush, and the unit re-bagged.

However well the inflorescences may have been selected, it never happens that all the florets of a unit open on the same day. Flowering proceeds progressively downwards (if the upper spikelets have been removed). It is possible that a point might be found when all the stigmas of a unit are simultaneously receptive and, theoretically, repeated pollination of the same unit might be avoidable, but under Aberystwyth weather conditions, pollen production to schedule cannot be depended upon, and therefore the rule for pollination is, that each female unit should be pollinated each day, as soon as, and as long as, fresh stigmas are produced and whenever over this period pollen is available.

Results.

It is unnecessary here to give any detailed results, but the following list of crosses which have successfully been made by the method described above will indicate its wide applicability. To avoid repetition, where reciprocal crosses have been successful in inter-generic and inter-specific crosses the + sign is used instead of the × sign. In some cases, the cross has been attempted only in one direction.

Lolium perenne	×	*L. perenne*
L. perenne multiflorum	×	*L. perenne multiflorum*
L. perenne	+	*L. perenne multiflorum*
Festuca rubra	×	*F. rubra*
Lolium perenne	+	*Festuca rubra**
Festuca arundinacea	×	*F. arundinacea*
Festuca pratensis	×	*F. pratensis*
Festuca arundinacea	+	*F. pratensis*
Lolium perenne	+	*Festuca pratensis*†
Lolium perenne	+	*Festuca arundinacea**
Festuca rubra	+	*Festuca arundinacea**
Festuca arundinacea	+	*F. gigantea*
Festuca pratensis	×	*F. gigantea*
Festuca ovina	×	*F. ovina*
Arrhenatherum elatius	×	*A. elatius*
Dactylis glomerata	×	*D. glomerata*
Phleum pratense	×	*Ph. pratense*
Alopecurus pratensis	×	*A. pratensis*
Festuca ovina	×	*F. rubra*

General.

When proper precautions are taken, this method has been found to be absolutely reliable, and is quite safe for critical genetical work. The chief precautions to be taken are :

(1) All inflorescences which are not to be used should be cut back so that no free pollen is produced within the greenhouse.

(2) Pollinations should not follow soon after flowering.

(3) The house should be closed down some time before the work of pollination commences.

(4) The house should be as nearly draught-proof as possible during pollination.

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* In these three cases, only the seeds produced in one type of cross germinated. Those from the reciprocal cross failed.

† With *F. pratensis* as the female parent in this type of cross, the male used in one successful case was a hybrid, *L. perenne* × *L. perenne multiflorum*.

(5) Soft brushes should be used so that the pollen is not violently scattered during pollination.

(6) Units, both male and female, should be exposed only for the briefest possible interval.

(7) Each brush should be sterilised after each pollen, and a sufficient supply of brushes kept at hand so that the same brush is not used twice in the same day.

The Continuous Emasculation Method.

In 1921, a different method was used for *Phalaris* spp. It had been observed that flowering in *Phalaris arundinacea* under greenhouse conditions commences about 5—5.30 a.m., reaches its maximum about 6.15 a.m., and is completed for the day about 10.15 a.m. The corresponding times for *Phalaris bulbosa* are approximately 10 a.m., 11 a.m., and 1.30 p.m. It was further noticed that the anthers take an appreciable length of time to become fully exserted, and, that there is a further interval between full exsertion and dehiscence. These facts suggested that it might be possible in these and other species and genera where the usual method of emasculation is difficult, to pick off the anthers successively during exsertion and up to time of dehiscence.

It so happened that the species selected were particularly well suited for a proper test. Both the cross and the test were purely experimental since it was not known whether the two species could be inter-crossed even under the most favourable conditions. In the sequel it was found not only that the two species are in some degree inter-fertile and in both directions, but also that the hybrid progeny plants can be distinguished beyond any possibility of error from the selfed progeny of either parent.

The results obtained are shown in Table VI. Fertile seeds of *Ph. bulbosa* develop hard and stiff paleae of a greyish colour, while those of *Ph. arundinacea* become even a darker colour. The pal of florets where the ovary has not been fertilised in both species remain thin and white. Relatively very few coloured seeds were obtained from *Ph. bulbosa* as female parent, and from these a total of 14 seedlings were raised, only one of which proved to be a hybrid. The remaining seeds had, therefore, been produced either by selfing or by accidental inter-crossing with other plants of the same species.

TABLE VI.—*Hybridization results with* Phalaris arundinacea *and* Ph. bulbosa, 1921. *Continuous emasculation during flowering.*

Cross number.	Female parent.		Male parent.	Florets emasculated.	Seeds.*	Hybrid progeny.	Selfed progeny.
69	*Phalaris bulbosa*	×	*Ph. arundinacea*	247	2	1	1
70	do.	×	do.	?†	10	0	10
71	do.	×	do.	?†	4	0	2
72	*Ph. arundinacea*	×	*Ph. bulbosa*	166	?‡	1	1
74	do.	×	do.	288	270§	6	0
75	do.	×	do.	781	?‡	10	3

* Caryopses apparently more or less developed.

† One inflorescence used in each case, total number of florets not ascertained, probably 200—300.

‡ A great many showing signs of fertilization but impossible definitely to separate them into " heavy " and " light." All " seeds " incubated for germination.

§ Paleae in all " seeds " more or less coloured, and ovaries therefore presumably fertilised.

With *Ph. arundinacea* as the female parent, the great majority of the " seeds " became coloured, and it would therefore appear that fertilization had occurred much more readily when the cross was made in this direction. Very few of these seeds were, however, capable of germination, but out of a total of 21 seedlings 17 were hybrids.

The point of interest at present is the fact that in both cases some seedlings of the same species as the mother plant were produced, and that therefore the method as applied in this case was not reliable as a means of inter-crossing. Its great weakness lies in the fact that the

inflorescences must remain exposed during the whole period of emasculation, and this period, at least in the two species under discussion, is not a short one. It may be shorter in other species, but in the present species an odd floret has been found to open much later than the great majority, and the pollen from such a floret may result in self-fertilization. Moreover, if several plants of the same species are being dealt with simultaneously, the danger of accidental " illegitimate " inter-crossing is not inconsiderable. The method, therefore, is of doubtful reliability except where, as in the present instance, the " crossed " progeny can be definitely distinguished from those produced by selfing and by accidental inter-crossing.

Hand Pollination without Emasculation.

This method can be used with reliable results where the female parent is known to be male-sterile. If the results need not be absolutely critical, it may also be used where the female parent is of very low self-fertility. As a means of obtaining a maximum amount of seed with the highest possible amount of crossing, it may further be used in other cases, and it is not infrequently used for *Phleum*, when it is immaterial whether a plant produced is the result of selfing or of inter-crossing, so long as it is vigorous and of the desired type.

The Mutual Pollination Method.

Since this method simply involves the isolation together of the inflorescences of two or more plants, it may be employed in several ways. In its simplest form, two plants only are concerned, and in this case the fabric balloon already mentioned or some similar arrangement can be used. For more than two plants, some kind of tent is usually necessary.

Since this method of " inter-crossing " two plants has been considerably used in " practical " plant breeding, it is necessary to examine it somewhat critically in order that the limits of its application may be better defined.

Theoretical Considerations.

Some evidence has been obtained which suggests that in certain instances a difference of some days in date of flowering may effectively isolate two strains of the same species even when grown in close proximity to each other. This means that for the success of the present method it is essential that the two members of a pair of plants should come into flower simultaneously. It is further necessary that at least one member of the pair should be male-fertile and that the two should be inter-fertile.

Experimental. (Experiment B. 142).

In 1925 a number of plants of *Lolium perenne* were paired up in various ways with a view to producing by an easy method a number of inbred progeny families for comparison of vigour. The plants were situated in the open and therefore fabric balloons were used for isolation purposes. At the time of bagging, also, care was taken as far as possible to bring together inflorescences which were apparently at the same stage of development.

The Plant Material.

Both related and unrelated plants were used, but the breeding characteristics of each plant were either already known or have since been ascertained. Eight pairs of male-sterile plants were matched, mainly in order that the efficiency of the fabric used might be tested. Three other pairs consisted each of a male-sterile and a male-fertile plant. The remaining pairs were made up of plants which were fully male-fertile and to some extent self-fertile.

The Fabric.

As already mentioned, 8 pairs of plants consisted of male-sterile plants only. In these, therefore, no seed could be expected except as the result of pollen from outside penetrating the fabric. Actually, 18 seeds were produced by 2,980 spikelets, showing a contamination of 0.6 seeds per 100 spikelets. Since the lowest seed setting in the other pairs reached 100.8 seeds per 100 spikelets, this amount of contamination is quite insignificant in the present connection.

Male-sterile with male-fertile plants.

The 3 pairs of this type are those shown under cross numbers 343, 345 and 353 of Table VII. The two male-fertile plants, plants 43 and 69, are both of very low self-fertility, but are fully male-fertile and produce an abundance of pollen. Seed setting in these pairs was somewhat lower on the average than in those which consisted of male-fertile plants, but it was still high and therefore pollen distribution within the cage was satisfactory. In these 3 crosses there is no evidence of self-fertilization.

Male-fertile pairs.

Had the experiment been designed especially for the purpose of testing the mutual pollination method of inter-crossing, plant material which would give results more easily interpreted might have been selected. The very considerable number of genetical factors involved, however, gives some additional interest to the results. In 6 pairs only 6 plants were used, and the genetical constitution of each of these in respect of seedling types is known.

Results.

The actual results obtained are shown in Table VII side by side with those to be expected in the complete absence of self-fertilization. It is not necessary here to discuss or to describe the seedling types concerned. It should be pointed out, however, that " b " represents two genotypes which are phenotypically similar, while type " e " can only appear in the absence of two dominant factors.

Before proceeding to discuss the main results it may be mentioned that some of them go to show that as far as can be ascertained they are perfectly reliable. Thus plants 43 and 110(1)7, which do not come within the present group, but which are genetically similar to each other in respect of seedling types, gave results in very close agreement with theoretical expectation. Similarly, plants 43 and 112(1)4 are similar in respect of seedling type " d," and therefore in respect of this type the same result would be expected whether the seedlings were produced as the result of inter-crossing or of self-fertilization. Again, actual and theoretical expectation agree very closely. Exactly the same remarks are applicable in the case of seedling type " c " in cross 359, and here again the deviation from expectation is insignificant.

It may therefore be assumed that in the other cases the results can be applied, allowing for small divergences between " actual " and " expected ", to the estimation of the amount of self-fertilization that occurred. In the table, the seedling types in each cross which could not possibly appear as the result of inter-crossing are shown in heavy type. These, of course, do not show *all* the seedlings which have been produced by selfing, but they can be used as the basis of calculation, so that the proportion of the " selfed " seedlings in the other groups may be estimated. Thus in cross 355, in the absence of self-fertilization, only type " a " (normal, persisting, full green) seedlings would be possible. The single type " d " seedling could only have been produced by self-fertilization in plant 43, and since it represents 25 per cent. of the selfed progeny of that plant, the proportion of plant 43 selfed progeny of types " a " and " b " can be estimated. Similarly, from the figures for types " c " and " e " together with those for type " b ", after deducting for plant 43, the proportion of type " a " seedlings which are the result of selfing in plant 48 can be estimated.

The estimations for the other families have been made in exactly the same way, and it is therefore unnecessary to discuss the results in any detail. True to its known character of high self-sterility, plant 43 has given very few seedlings from self-fertilization, but the much more highly self-fertile plant 48 has contributed from selfing over 40 per cent. of the total seed produced in cross 355. In " crosses " where estimations for both members of a pair are possible, the lowest degree of selfing reaches 33 per cent. of the total seed produced.

It will be borne in mind that every effort was made in the first instance to match up the inflorescences of a pair of plants with a view to coincident flowering. It will also be noticed that the same plant was used in several pairs, so that it is highly improbable that in every case flowering failed to coincide fairly closely. There is, further, no question of incompatibility, since the same crosses have successfully been made by hand, while as shown by the male-sterile +

TABLE VII.—*Experiment B.* 142. *Pairs of perennial rye-grass plants isolated together for mutual pollination.*

Cross numbers.	Station numbers of pairs.	Number of spikelets enclosed.	Number of seeds per 100 spikelets.	Total no. of seedlings obtained.	Seedling types.										Estimated percentage of seedlings due to self-pollination of either parent.	
					Actual proportions.					Proportions expected with no self-fertilization.						
	A. B.				a	b	c	d	e	a	b	c	d	e	A.	B.
343	64+69	173	150.3	253	253	—	—	—	—	253	—	—	—	—	0	0
345	69+100(1)6	381	100.8	368	368	—	—	—	—	368	—	—	—	—	0	0
353	43+110(1)7	229	221.4	401	221	72	—	108	—	226	75	—	100	—	—	—
355	43+48	341	230.8	732	576	**50***	**87**	**1**	**18**	732	—	—	—	—	1	44
357	43+112(1)4	281	170.5	457	217	123	—	117	—	257	86	—	114	—	0	42
358	48+112(1)4	351	198.3	641	411	131	**60**	**26**	**16**	483	161	—	—	—	40	16
359	48+112(1)29	316	216.8	590	365	**40**	153	**23**	**9**	442	—	148	—	—	17	16
362	112(1)4+112(1)8	257	184.8	451	202	205	—	**44**	—	254	197	—	—	—	39	?
365	112(1)8+112(1)33	182	290.1	487	330	131	**23**	—	**3**	365	122	—	—	—	?	18
367	43+85	146	117.8	163	163	—	—	—	—	163	—	—	—	—	0	?

* Figures in heavy type indicate seedling types which could not have been produced by inter-crossing.

male-fertile pairs, pollen distribution within the containers was satisfactory. It is, therefore, impossible to avoid the conclusion that when either one member or both members of a pair of plants enclosed together for mutual pollination are at all highly self-fertile, a very high proportion of the total seed produced may be the result of self-fertilization.

On the other hand, where the plants are male-sterile or of low self-fertility, only a relatively small proportion of the seed may be expected to be due to self-fertilization. Thus in crosses involving plants 43, 69, and 85, in Table VII, there is little evidence of selfing in these plants.

For critical work the method obviously fails completely, and, as in the case of the method of hand pollination without emasculation it can safely be used only where it is immaterial whether a particular progeny plant is the result of self-fertilization or of inter-crossing.

STRAIN-BUILDING.

As used in the present connection the term " strain " presupposes a certain amount of conscious selection. Its application, however, is still very wide, since on the one hand a single plant may be selected as the basis of a strain, and at the other extreme, an entire population from a particular source may be taken.

In the discussion of strain-building, certain illustrative instances will be given. These are not intended to represent ideal methods, but they have been selected in order that various aspects of the problem may be discussed, while certain mistakes and errors of procedure which were almost inevitable while experience and knowledge were being gained can also be emphasised.

For present purposes, strains may be considered to be of two main types, (1) single-plant strains, and (2) multiple-plant strains. The first type is based exclusively upon a single selected plant with subsequent strict breeding within the line ; the second may be based upon two or more selected plants.

THE SINGLE-PLANT STRAIN.

Whether it is possible to produce a strain based upon a single selected plant depends upon the characteristics of the plant itself, and whether it is further desirable to establish such a strain will depend upon whether the object in view can best be attained by this method. The first essential in any case is that the plant should be in some degree self-fertile, and that the progeny plants so produced should be (1) self-fertile ; (2) inter-fertile with the mother plant, or (3) inter-fertile with each other.

FERTILITY TYPES.

Male-sterile Plants.

Male-sterile plants have been found in perennial rye-grass, Italian rye-grass, cocksfoot, timothy and tall oat grass. Morphologically they do not differ in any essential respect from other plants of the same species. All such plants so far tested have been found to be fully female-fertile, but although the anthers may be, and usually are, normally exserted, they are slender, yellow or greenish in colour, and do not dehisce. Such plants obviously cannot be used for the establishment of single-plant strains.

Partially Male-sterile Plants.

This type differs from the preceding one in that some of the anthers show slight dehiscence, and a small amount of pollen may therefore be liberated. Such plants are not necessarily self-sterile. Thus, perennial rye-grass plant 399(1)11 liberates only a trace of pollen and yet in the only year tested, 1927, it gave the high yield of 143 seeds per 100 spikelets from self-fertilization. Such a plant, despite high male-sterility, may still be regarded as the potential basis of a single-plant strain.

Fully Male-fertile Plants.

Fully male-fertile plants are not necessarily self-fertile, but it is difficult to prove that any plant which produces an abundance of pollen is absolutely self-sterile. A single test or even a few tests, as shown by the results given for perennial rye-grass plant 43, may give negative results, and yet the plant may be capable of setting a few seeds from self-fertilization.

Within any one grass species, the variation in degree of self-fertility may be from complete or nearly complete self-sterility to complete or almost complete self-fertility, but it is yet probable that the average degree of self-fertility differs considerably from one species to another.

Inter-fertility.

So far, very few cases have been found in the herbage grasses where sister or otherwise related plants are completely inter-sterile, so that it would appear that this difficulty will rarely be encountered in the production of single-plant strains.

STRAINS IN THE MAKING.

Two examples will be given to illustrate two distinct methods of procedure with regard to the establishment of single-plant strains, and in order that difficulties encountered and mistakes made may be discussed.

Perennial Rye-grass Strain 48.

Actually, this line of breeding has never reached the dignity of a strain since it was abandoned at a fairly early stage.

The aim in this case was to explore the possibility of producing a homozygous perennial rye-grass strain by repeated selfing of selected plants in successive generations. This line of investigation was suggested by the fact that it has been possible to produce maize strains of this type which are of economic importance even though they may not themselves be particularly productive.

Plant 48, upon which it was intended to base this particular line, was found growing " wild " on old semi-derelict garden allotment ground. Individually, it was particularly vigorous, and it was later found to be also relatively highly self-fertile. It, therefore, seemed in these respects to be suitable for use as the basis of a single-plant strain.

From self-pollination, however, four distinguishable seedling types were obtained, two of which were incapable of surviving, while very few of the third group could proceed beyond the seedling stage. Thus about 50 per cent. of the total number of seedlings produced were lost. The earliest results obtained appeared to indicate that those which survived were more vigorous than the average for selfed progeny in perennial rye-grass, but subsequent results, including those to be referred to presently, showed that actually they are of about average vigour.

Three generations of seedlings were ultimately obtained in the same season and were planted out with the intention of collecting productivity data by cutting and weighing, but this particular experiment, B. 169, was only used for observation purposes, and the results so obtained strongly supported the actual data derived from experiment B. 150 for first and second generation progeny. The full results for this experiment, in so far as they touch upon inbreeding, are given in Table VIII, p. 29*, but for the moment we are only concerned with the three families at the bottom of the table. The L_1 family derived from plant 48 shows a loss of vigour as compared with the same plant out-crossed to plant 43 of 66 per cent., while the L_2 family from the L_1 plant 48(1)21 showed very little difference from the L_1, although observation both on the plants from which the data were collected and others in an area adjoining indicated that there actually was an appreciable difference between the two families. The difference between the L_1 family and the other L_2 family produced by inter-crossing two L_1 plants was certainly no greater than indicated in the table. In any case, the difference between these three families is so small that under the conditions of the experiment it cannot possibly be statistically significant.

* This experiment will be more fully described and discussed under another section.

It would appear that in this particular line (and very similar results have been obtained in other lines of perennial rye-grass) the great loss of vigour occurs in the first generation from selfing, and that there is the possibility of a slight recovery in the second generation if selected plants are inter-crossed. Such recovery is, however, extremely small, and is of no practical value since the plants obtained are still extremely poor.

The third generation plants as compared with those of the first and second in experiment B. 169 appeared to be again slightly less vigorous, but at this point the experiment was stopped owing to the fact that no purposeful selection was possible.

There is no reason to believe that this is an extreme case for perennial rye-grass, since results published elsewhere (Jenkin, 1927), show on the average a loss of vigour from selfing of over 60 per cent. The same result would, however, not be expected in the case of all perennial rye-grass plants, much less so in all plants of other species. At the same time these results indicate a general danger.

A plant may be selected on its own individual merits—particularly on vigour and desirability of type. Excessive vigour as compared with other plants of the same species grown under similar conditions may in itself be a symptom of greater heterozygosity, and therefore selection on vigour may also be in many cases the selection of the most heterozygous even in the first instance. A " desirable type " again has no meaning beyond what the selector may have in his own mind. It certainly does not necessarily mean a type which represents a homozygous condition. Consequently a selector in choosing a plant on its own merits may be doing so on qualities which are necessarily unstable. Whether loss of vigour then be great or small, if he persists from generation to generation in selecting on the same basis, namely, for the greatest possible vigour and the closest approach possible to that of the original plant, he may be unable to make any appreciable progress towards the homozygous strain.

On the other hand, if his first consideration is the production of a homozygous strain, he must abandon his ideas of the importance of both vigour and type and select mainly towards extremes. The ultimate strains so produced may then be entirely different from the plant from which he started, and although several homozygous strains may be evolved, it is quite conceivable that none of them will at all fully represent the original plant. It will be borne in mind that plant 48 was found in an absolutely unstable habitat and that it might, therefore, be expected to be highly heterozygous. Even in the most stable habitat this species has not been found to have produced a homogeneous phenotype, but in other species such as cocksfoot and timothy, this has been approached much more closely. If, then, the original selection were made within a well defined ecotype, the dangers already referred to would still exist, but in this case they would probably be at their minimum.

Timothy Strain 309.

This line represents an entirely different method of procedure for the production of a single-plant strain. In fact, it represents the purely practical aspect rather than the theoretical method adopted in the case of the perennial rye-grass strain.

The plant selected in this case was grown from seed collected near Aberystwyth. It differed from the great majority of plants produced by ordinary commercial seed in that it was later flowering, more dense, and more leafy. It is, however, doubtful whether it was truly indigenous. More probably it was an " escape " (several generations removed) from cultivation and was able to persist owing to its divergence from the average type.

From amongst its selfed progeny, 17 of the best plants were selected, broken up into plantlets and pooled with a large number of the propagants (vegetative propagation) of the mother plant to form a seed production area. Seed production from this area was very decidedly low from season to season, presumably owing to the fact that the population consisted very largely of the propagants of the mother plant. The plants raised from seed thus harvested were relatively very uniform and produced seed much more freely. In the following generation the plants obtained showed a considerably greater diversity of type. Even then, however, the range of variation was relatively narrow and the general type could be maintained from generation to generation so long as no out-crossing occurred, and mass selection in the stock seed production areas was practised.

This strain represents an entirely different conception of what is required of a single-plant strain. In this case, no attempt was made to achieve homozygosity. All that was required was that the resulting strain should be relatively homogeneous in type, while it also remained sufficiently vigorous to be superior to other existing strains at least for certain conditions. The method of procedure is obviously open to severe criticism on theoretical grounds. In particular, evidence obtained from the study of a single individual and its first generation progeny was accepted as sufficient, and nothing was done at this stage to select the progeny plants on their own breeding characteristics. It is, therefore, probable that in selecting for vigour and type the most heterozygous plants were selected and pooled with the mother plant, with the result that after the first generation from pooling a greater diversity of type appeared.

From the practical standpoint it might be argued that the basis of the strain was too narrow—that a single plant as the basis of a strain is insufficient to maintain a high degree of vigour in later generations from inbreeding. Whether this be so or not probably depends almost entirely upon the characteristics of the plant itself. Perennial rye-grass, plant 48, could certainly not have been advantageously employed in the same way, and it is further possible that no plant of whatever species can usefully be employed in this way if from self-pollination a great loss of vigour occurs.

MULTIPLE-PLANT STRAINS.

The multiple-plant strain has the initial advantage over the single-plant strain that there is less danger of loss of vigour from inbreeding and, theoretically at least, the greater the number of plants used the less the danger from this source. On the other hand, the greater the number of plants used, the greater the danger of lack of uniformity in the progeny, and of divergence from the original type in subsequent generations.

Source of origin in relation to Strain-building on the Multiple-plant Basis.

The importance of source of origin has already been discussed to some extent. The habitat from which the perennial rye-grass plant previously referred to had been taken was extremely unstable, and under such conditions any type of plant might have some chance of persisting. The combinations possible by inter-crossing would, therefore, be the widest possible, but the inter-gradation of types in this species is so great that two plants which are very different genetically might be approximately similar in appearance. Thus two plants from such a habitat might be selected on general appearance and inter-bred, and in the second generation give rise to a great variety of types.

Transitional zones between two stable habitats inhabited by a particular species may easily produce plants which would give similar results. Thus, as shown by Stapledon (1928) cocksfoot is at home both in the heavily grazed old permanent pastures and in thickets. The types which occupy these two positions are however widely different, but in the transitional zone a great diversity of type occurs. Plants from this area may therefore be expected to be much more heterozygous than those from the stable habitats on either side. Many such plants are very vigorous, and often apparently of a desirable type, but owing to the unstable nature of the habitat in which they have been produced, they require very careful study before they can be used as the basis of a strain.

Plants of the same species produced in the more stable habitats are both themselves more homogeneous in type and give rise to more homogeneous progeny. Thus timothy rootings taken from old heavily grazed pastures on heavy land almost without exception gave rise to plants which differed from each other only within relatively very narrow limits, and their progeny showed no serious transgressive segregation. Similarly, another distinct type of timothy was produced from seed collected on the chalk downs where the soil was light and dry, and the pasture was only very lightly grazed. The difference between the two types was mainly a difference in growth habit, but this was very definite. These two types are also inter-fertile, so that the difference appears to be due to " natural selection " acting on an original general type and resolving it into its constituents.

This does not mean that any plant taken from any particular habitat is absolutely homozygous. Under such stable conditions two different stresses are operative. On the one hand, the uniform conditions tend to favour one particular growth type, although a certain range may be allowed. On the other, vigour is of great importance in competition, and therefore in connection with persistency, and the added vigour due to greater heterozygosity works somewhat in the opposite direction, unless it so happens that a type which exists only in the heterozygous condition is also the one which best suits the habitat. A relatively homozygous plant under these conditions must in any case be also a vigorous one. If, therefore, material is selected from such a habitat, there is at least a chance that a plant which is relatively vigorous will also be to a considerable extent homozygous.

It is therefore evident that it is possible to be too ambitious with regard to vigour, and that selection of the second best may lead to more satisfactory results.

It is further quite clear that in selection work, the origin of the plant is of surpassing importance, and selection should not be on absolute characteristics, but more particularly in relation to the general type occupying a particular habitat. Thus, it would be almost hopeless to make any attempt to select a true persistent perennial rye-grass type suitable for permanent pasture purposes from a population grown from ordinary commercial seed, owing to the fact that the latter has been grown under conditions which are inimical to the persistent pasture type for so many generations that apparently it has been eliminated completely. Yet the pasture type still exists in abundance in old pastures, and here obviously must improved pasture strains be sought.

Inbreeding in Relation to Retention of Vigour.

Any system of purposeful breeding must involve a certain amount of inbreeding, but in connection with multiple-plant strains a point of great difficulty arises, namely, how wide the basis must be or how narrow it can be without serious loss of vigour from inbreeding.

To solve this question very elaborate and very extensive experiments would be required, particularly since, as has already been shown, in one case a single plant may appear to be adequate, while in another case it may be hopelessly inadequate. The results given in Table VIII obtained for perennial rye-grass in experiment B. 150 are not intended as a solution of the problem, but they represent in skeleton form the type of results which are required for a better understanding of the position. In this case two original perennial rye-grass plants are mainly involved. These were inter-crossed and a certain amount of inbreeding of various kinds followed.

TABLE VIII.—*Inbreeding in perennial rye-grass. B.*150.

Parents of family.	Status of family.	Method.	Number of plants.	Relative produce per plant.
48 × 43	Unrelated	Crossed	25	100
48(1)22 × 43	$L_1 \times P_1$*	,,	25	94
112(1)23 × 48(1)22	$F_1 \times L_1$	,,	24	78
48(1)21 × 112(1)23	$L_1 \times F_1$	,,	25	74
110(1) 8 × 43	$F_1 \times P_1$	,,	20	75
112(1)23 × 43	,,	,,	25	66
111(1) 3 × 43	,,	,,	15	64
110(1)36 × 43	,,	,,	25	60
112(1)23	$F_2 = L_1$	Selfed	24	44
111(1) 3	,,	,,	24	38
48(1) 21 × 48(1)22	$L_1 \times L_1$	Crossed	25	37
48	L_1	Selfed	24	34
48(1)21	L_2	,,	25	32

* Unrelated.

However inadequate they may be, the results are at least interesting, and a few particulars of the experiment are therefore necessary. All the progeny families were treated exactly alike, and the lots were planted in the experiment on a single-plant replication basis, so that each family as far as possible was represented over the whole area. In this way the effect of soil differences was at least largely eliminated. The plants in the seedling year were merely kept under observation, but in the second year each plant was cut four times and the herbage weighed independently. All families, except two, were originally represented by 25 plants, but in some cases a single plant missed. Taken at their face value, the results fall fairly easily into four definite groups.

The greatest yield was obtained from the cross between the two unrelated plants 48 and 43, but that given by an $L_1 \times P_1$ cross (again unrelated plants) falls only a short distance behind, in spite of the fact that the L_1 plant 48(1)22 was far less vigorous than its parent plant 48.

The next two families represent a side-cross between half-sister plants ($L_1 + F_1$), and these show a very definite decrease in productivity amounting to nearly 25 per cent. as compared with the main out-cross.

One family of type $F_1 \times P_1$, representing a direct back-cross, also shows a 25 per cent. decrease, but the three remaining families in the same group indicate a loss of vigour of about 37 per cent., thus giving an average for the group of about 33 per cent.

For present purposes, all the L families may be taken together, and these show an average loss of vigour of about 63 per cent.

No definite generalisations can be made until these results have been supplemented, although the relation between $P_1 \times P_1$ and L_1 results obtained in this case is almost exactly the same as the average previously obtained for similar relationships (Jenkin, 1927). It is therefore difficult to avoid the conclusion that the other results, particularly since they appear to be so definite, also indicate the type of result to be expected from these two forms of inbreeding.

Tentatively it may then be suggested that the loss of vigour which occurs as the result of selfing is only the extreme expression of a condition which results in greater or less degree in all forms of inbreeding with ordinary material, according to the degree of relationship existing between the two plants concerned. In perennial rye-grass, loss of vigour from selfing is extreme, and consequently the results from other forms of inbreeding will also be relatively pronounced. Where in the case of any particular plant this loss of vigour from selfing is relatively very small, other forms of inbreeding may have no very appreciable effect.

Applied provisionally and in the absence of more adequate data to the question of the number of plants required as the basis of a strain in order that vigour can be maintained, this would mean that the number should vary according to the peculiarities of the plants concerned. In one case, already discussed, it is fairly obvious that two plants are insufficient, while in another case no serious deterioration occurred in a strain based upon a single plant.

SOME MULTIPLE-PLANT STRAINS.

Timothy Strain 310.

In main characteristics this strain is very similar to strain 309 already described, but it has been found to be superior to the latter in certain respects.

The two strains were started almost at the same time, but by different methods. Strain 310 is based upon seed collected on the rough headland of an old pasture field in Dorset, but in collecting the seed, two rather serious mistakes were made. The first mistake was to bulk the inflorescences together and to regard them as a single unit: the second, in effect, although at the time unconsciously, to regard this rough headland as a stable and uniform habitat, whereas it really graded from the hard-grazed open pasture to the rough tangle in the hedgerow. The seedlings grown from this bulk varied very considerably in type, some of them agreeing very closely with material collected later from other old pastures. The others again varied amongst themselves, but they could be fairly easily grouped into sub-types. Many of these plants were selfed and as a result of an examination of the progeny plants and their parents, four original plants and selected plants from amongst their L_1 families were pooled as the basis of the new strain. All these selected plants were relatively very uniform in type, and subsequent breeding and

increase has shown that the general type is fairly easily maintained by means of mass selection.

In pooling the plants, however, a third serious mistake in the history of this line was made. The four basic plants were entirely used up and their identity lost, whilst representative plantlets of each should have been kept in reserve in order that their breeding characteristics might be more thoroughly investigated and so that at any time this strain could have been re-started from exactly the same point.

Timothy Strain 326.

In this case a modified form of mass selection was used. More than 40 plants all very similar in general type, but differing considerably in detail, were pooled for seed production. At the time, this method appeared to be justifiable owing to the fact that the general type was easily recognisable, while all the plants were derived from stable and uniform habitats. In fact, however, it has later been realised that this meant the inclusion of plants which were heterozygous for susceptibility to rust disease, and this fact has been a constant source of trouble. The strain is now being re-established on a narrower basis after a more thorough study of the individual plants to be used.

Perennial Rye-grass Strain 1412.

In 1920 a single inflorescence of perennial rye-grass seed was collected in a very old pasture field in Pembrokeshire. This yielded five seedlings, three of which were very vigorous and very uniform in type. One of these three was self-pollinated, while the remaining two were inter-crossed. The three plants were then multiplied vegetatively and they, together with selected plants from amongst their progeny, were pooled for seed production. Since it was felt, however, that by using only three plants and these very closely related, the basis of the strain would be dangerously narrowed down, a very minute representation of four other unrelated plants of very similar type was added.

This strain has so far given highly satisfactory results, but through imperfect isolation it became contaminated and it was necessary to re-establish the strain. The three original plants were still available, while two other very attractive plants of very similar type but of different origin were now also available. These two plants were male-sterile, and they therefore appeared to be ideal material for the introduction of new " blood " into the strain, since it had been observed that the progeny of such plants were also in some degree male-sterile. This would mean that in the first instance they could act only as female parents and that thereby the proportion of outside " blood " would be kept very low. Consequently, these two plants were inter-crossed with one of the original three, and from amongst their progeny 28 plants were selected. With these, 46 plants obtained again by inter-crossing the three original plants and 51 plants selected from the old strain were pooled for seed production. Thus about 20 per cent. of the total population now consisted of out-crosses to plants of similar type, so that theoretically the two outsiders were represented by 10 per cent. of the gametes the population could produce. This re-established strain has also given good results, and the plant type is very uniform and similar to the original, except that an occasional plant of an extreme type sometimes appears. It has now been realized, however, that while out-crossing to the extent indicated appears to have been fully justified, it was a mistake to use male-sterile plants for this purpose.

Male-sterile plants, as a rule, are particularly vigorous, and since those so far met with are also fully female-fertile in a population they produce an undue proportion of the total seed. Moreover, although the mode of inheritance of male-sterility has not yet been fully studied, it is evident that for some reason male-sterile plants produce a higher proportion of male-sterile than of male-fertile progeny. These two facts combined, under ordinary conditions of strain increase where no selection of any kind is possible, tend to an undue increase in the proportion of male-sterile plants in the strain. The real danger of this lies, not in the possibility that thereby the strain may be brought to a high state of sterility, but in the fact that a population of this kind, isolated by distance only, positively invites contamination from stray pollen since its own pollen production is inadequate. Thus the identity of a strain might be rapidly and completely lost.

This strain is again being re-established upon the basis of the three original plants, but some other plants of similar type and breeding characteristics and which are male-fertile are being used.

Strain-Building in Italian Rye-grass.

If sown very early in the season, Italian rye-grass may behave as an annual, but if sown about July (or if after having been sown early its development is checked) it is at its best in the following season. Individual plants, however, differ greatly in rate of development and time of reaching maturity, so that even here we are not dealing with absolutely uniform material. At the most, however, plants remain really vigorous only for two seasons, and since they transplant very badly they cannot successfully be studied in clones. As a rule, however, plants can be perpetuated for a few seasons by breaking up and rejuvenation, so that it is possible to keep parent plant material in reserve while their progeny are being tested.

For these reasons, economic breeding and strain-building in Italian rye-grass must be conducted on a somewhat different system from that which can be applied to the more perennial species. Selection of the individual is necessarily both first and final, but if a particularly rapid growing type is sought for, it is advisable to sow early in the season, since such a type is at its best in the first season, while for the selection of a type to be of greatest use in the second season, late planting is preferable.

As in the case of perennial rye-grass, there is great loss of vigour from selfing, so that except as a means of ascertaining whether a plant is heterozygous for defective seedling characters this method is of relatively little use. The further study of the selected plant is therefore made by inter-crossing (diallel crossing) and their breeding characteristics ascertained by a study of their crossed progeny. It is then possible to return to the original parents and produce further F_1 families which can be pooled for seed production.

When the strain has reached this stage, the generations follow rapidly after one another, since only a single crop of seed can be usefully taken. This means that the real basis of the strain is changed in successive generations, and it is necessary for " stock " purposes to practise heavy roguing in each generation.

In practice, however, further refinements have been introduced. Apart from roguing in a " stock " seed production area (by which means inferior types are rejected), certain plants of outstanding merit are marked down, and open-pollinated seed of these is harvested independently.* In the next generation area, these marked plants are represented by families which may together form 50 per cent. of the whole population, but the identity of each family is kept so that it may be possible to reject an entire family if it is found to be inferior to the average.

It is further possible by carrying seed over from one season to the next to have the strain established in two seasons, so that for multiplication stock seed may be available in every season. Further, the progeny of the selected plants of one year can be thus compared with those of the plants selected for individual harvesting in the next season, and the strain can thus be kept relatively uniform in type.

GENERAL NOTES ON PROCEDURE.

Preliminary Procedure.

Reference has already been made to the study of individual plants by means of their progeny. An interesting point then arises—whether a plant which, on selfing, is found to give defective seedlings should be rejected without further consideration. Theoretically, if it were possible to inbreed over successive generations the proportions of heterozygous plants of this type in a strain based upon a single plant would automatically be reduced to an inconsiderable minimum. It may be doubted, however, whether theoretical expectation is here directly applicable to practice, since the heterozygous plants may, on the whole, be more vigorous than the homozygous dominants, and will therefore be perpetuated in greater proportion than that allowed by theoretical

* It might be suggested that these should be selfed, but the loss of vigour from selfing is so great that purposeful selection of the progeny becomes impossible.

expectation. On the other hand, if the strain is based upon say four plants, and only one of them is heterozygous for such a character, the chances are greatly against its having any serious effect on the strain as a whole, and an occasional defective segregate is a matter of no great importance. The danger obviously becomes greater as the number of such factors increases either in the case of any one of the plants, or separately in different plants, so that a limit must be set to the proportion of such plants to be included.

Progeny Tests of Potential Basic Plants.

When a strain is to be established, potential basic plants are, as already indicated, first selected on their own individual merits, with regard to vigour and with regard to type in relation to the purpose for which the strain is intended. In selecting for any particular purpose also, the origin of the material is kept in mind, and material from unstable habitats is used with considerably greater caution than that from more stable habitats.

If the plants selected are fairly highly self-fertile and there is little loss of vigour on selfing they can be directly compared by means of their progeny. If not, it is necessary to inter-cross all the plants within a group in every possible direction, so that from say 8 plants 28 different families are possible. Ideally, representative plants of these families should again be inter-crossed in order that the F_2 families might be compared particularly with regard to the appearance of unsatisfactory plant types. This is necessary since two plants which are very similar to each other may give very uniform progeny in the first generation, but transgressive segregation of a serious kind may yet occur in later generations. When this has been done, it is possible to select those plants which have been proved to be the most satisfactory parents as the permanent basis of the strain, and plantlets of these should be kept in reserve, while they may also be increased vegetatively and blended with their progeny for seed production.

Self-fertility in Relation to Strain-Building.

When a strain is to be produced on a single-plant basis, a certain degree of self-fertility is essential. This quality is also extremely useful in order that some of the breeding characters of provisionally selected parent plants may quickly be ascertained. Otherwise, its utility in general breeding work may be seriously questioned. Even for genetical work with perennial ryegrass self-fertility gives little assistance, except with certain individual plants, since not infrequently germination is poor while the seedlings are also weak.

In strain-building another serious aspect of the question arises. When a plant which is at all highly self-fertile is enclosed with others for mutual pollination, as may happen when a number of plants are used for the establishment of a strain, a high proportion of the seed produced may be due to self-fertilization. The plants derived from these seeds will have a depressing effect upon the productivity of the first generation, although if they are inter-crossed with unrelated plants they may yet give good results in the following generation. If (as remains to be proved) plants which are highly self-fertile in the presence of others when enclosed are also similarly highly self-fertile under field conditions and selfing leads to loss of vigour, a proportion of the plants will always be lacking in vigour so long as the influence of such plants remains in the strain. If all the basic plants of a strain are similarly highly self-fertile, despite the fact that when inter-crossed they give satisfactory progeny, the amount of self-fertilization that may happen in each generation may be so great that the highest potentialities of the strain are never realised.

Thus it would seem that for multiple-plant strains, a high degree of self-fertility in the basic plants may be positively undesirable, and that the ideal plant is that which produces an abundance of pollen, but which is at the same time highly self-sterile.

The data used in writing the present paper have been collected over several years, and during this period I have been assisted by various people. Acknowledgments are in particular due to my colleague, Mr. A. R. Beddows, B.Sc., who has in recent years rendered great assistance in connection with hybridization and self-pollination work.

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METHODS AS APPLIED TO COCKSFOOT GRASS (*DACTYLIS GLOMERATA* L.) AND REMARKS AS TO TECHNIQUE IN GENERAL.

BY

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INTRODUCTION.

Cocksfoot, to a greater degree than any other grass under study at Aberystwyth, occurs as more or less well-defined habitat races or ecotypes, and although of course normally cross-pollinated it is more highly self-fertile than perhaps the generality of herbage grasses.

In my work on cocksfoot I have, therefore, concerned myself rather with type or growth-form as such than with the behaviour of single plants as such. As a consequence of this, the methods I have adopted in selection and breeding have differed somewhat from those described by Mr. Jenkin.

Preliminary Study and Collection.

The methods of collecting plant material have been similar to those described in the previous article—except that generally larger quantities of seed have been collected per habitat and larger populations dealt with. By 1921 I had collected a vast " museum " of living material and had classified my species into about a dozen main " growth-form " types (Stapledon, 1928), based on an intimate study of individually spaced plants.

Testing the Agricultural Potentialities of the Types.

Having categorized my species, and having become acquainted with my types, the next necessity appeared to be to ascertain as far as possible—still confining myself to single plant material—the behaviour of these types under conditions which might be expected to approximate closely enough to those obtaining on a sward, to give some index of the reactions of the aforesaid types to sward conditions proper.

I therefore decided to subject my types as such to experimental scrutiny. I regarded the ordinary Danish (commercial) hay type as a standard for comparison. In deciding upon the treatments to which to subject my types I obviously had to be guided by an acquired " sense "—the outcome of at least three years intimate experience of cocksfoot and of a good many years apprenticeship to the study of grassland. Sooner or later, no matter what his technique, every plant breeder has to rely to a considerable extent on this acquired " sense ".

My scheme of experimentation in the first place entailed the matching-up of a large number of plants (each as truly representative as possible of its type). Obviously so far I was not in the least interested in the genetical make-up of my plants, all I wanted was enough plants of each of my " growth-form " types to give me sufficient material to compare and contrast the several types as such—and to give, moreover, sufficient material for replication and collection of quantitative data.

Closely Spaced Plant Rows.

Each of the individual plants chosen to represent a particular type was broken up into four ; the propagants were mixed indiscriminately and planted 9 inches apart in rows 20 feet

long and 1 foot 6 inches apart. The rows for each type were replicated and the experiment divided into two sections. One section was cut for hay and aftermath each year, and the other was cut monthly on a pasture basis. This experiment was kept in being for four years.

Tiller Rows and Tiller Beds.

In order to set up these experiments each of the plants was broken down to small tiller units. The " type " rows were made by planting representative tillers close together. The " beds " were made by dibbling in the tillers at a distance of about 2 inches apart in all directions. Both the tiller beds and the tiller rows were replicated and, as in the case of the spaced plants, were subjected in part to a system of pasture cuts and in part to a system involving only the taking of hay and aftermath.

Some of the " pasture " beds and rows were cut monthly, and others fortnightly.

Palatability Tests.

Tiller beds as well as being set up in the Gardens were also established on a field at the Farm, and these latter were grazed by sheep (under control) at regular intervals, and careful notes were taken as to relative palatability.

Sown Beds.

It was of course at this stage quite impossible to sow out beds with " true-to-type " seed of each of my types. Beds were, however, sown with Danish commercial seed, with seed from Akaroa (New Zealand), and with seed collected from thickets in the neighbourhood of Aberystwyth. A study of a large population of single plants from each of these three aggregate sowings accurately showed the types which most abundantly contributed respectively to Danish, to Akaroa, and to Aberystwyth " thickets ". These sown beds were subjected severally to a hay and aftermath system of cutting, to cutting monthly with a garden mowing machine, and to controlled grazing by sheep.

Lessons from the " Growth-form " Tests.

The evidence from all these experiments seemed to point in the same direction, to the effect that the multi-tillered types with a high proportion of barren tillers to total tillers were the most persistent and the most leafy. There was also strong evidence to suggest that the very narrow leaved forms were not as palatable to sheep as broader leaved forms. Further, there was evidence to suggest that the narrower leaved forms were perhaps on the whole more persistent than the broader leaved forms—some of the broader leaved types were, however, quite remarkably persistent.

As the result of these trials (which all through had been supported by weighing the produce of the several cuts) I felt fully justified in selecting from my types one which I considered ideal for hay, one as ideal for dual-purposes (i.e., hay and pasture), and two which I regarded as likely to be ideal for pasture.

I should mention that contemporaneously with these trials Professor Fagan (1929) had been investigating the chemical composition of representative types, and had reached conclusions which seemed to justify the assumption that high leaf yield and a high proportion of leaf to total yield were of supreme importance. In selecting my " ideal " types I therefore attached much importance to leafiness, much importance to persistency, and much importance to sustained productivity, but on the score of palatability rejected the very narrow leaved types.*

Seed Producing Propensities of the Several Types.

No matter what ideal the plant breeder sets before himself in other respects, he cannot ignore seed producing propensities. At an early stage of my experiments I therefore took steps to ascertain the comparative seed yielding ability of my types—certain types, otherwise excellent,

* It must not necessarily be supposed that narrow leaved grasses are always at all times and in all places unpalatable, or that in all species the strains with the narrowest leaves are the least palatable within the species. The matter is, however, one deserving very close attention.

had to be rejected on this score. The net result of all the experiments was, however, to show that excellent pasture types existed which were quite compatible with reasonably good seed production. It was none the less apparent that in connection with the more leafy pasture types it would be necessary to study closely the technique of seed production ; this is not, however, a question that can be usefully discussed in the present connection.

General Discussion of Methods appropriate to the Breeding of Cocksfoot in particular and of Herbage Plants in general.

I had now, to my own satisfaction at all events, established the fact that certain types of cocksfoot—types abundant in Nature—were vastly superior to the types obtainable from the ordinary seed of commerce—at least for a large number of conditions and purposes. I had ascertained this further fact, that a population of plants obtained from say Danish commercial seed would not be likely to contain 14 per cent. of plants representing my four select types ; while a population of plants established from Akaroa seed would be unlikely to give at the very most more than 50 per cent. of the plants I wanted. Here then was a very interesting and a highly significant state of affairs. I had only to " breed " a strain of cocksfoot giving about 70 per cent. of my types, and in the economic sense of the word I should have achieved something of immediate practical importance.

Let it be very clearly understood that in the improvement of herbage plants, even if the " improved " product does contain a considerable admixture of unimproved material, no positive harm is done. The unimproved admixture is likely to be as palatable to stock as (or perhaps more palatable than) the " improved " material, and is likely to be eaten in proportion to its productivity as long as it lasts. Very different of course is a commodity which has to go through a process—such as being baked into a loaf or woven into a fabric : here the product must necessarily be valued as one entity, and any dilution by inferior and unimproved elements will completely counterbalance or mask such excellencies as it may possess.

This point seems to me to demand considerable emphasis, for in a sense it raises a new issue relative to plant breeding. It comes to this, that in regard to certain crops—herbage plants are an outstanding example—the economic plant breeder may hope to achieve valuable results without necessarily becoming a slave to the idea and to the ideal of genetical purity. It may often be legitimate, in relation to practical usefulness, also to look at purity relatively and on a percentage basis. The herbage plant breeder (and I have little doubt the plant breeder of some other species also) will have done much if he can produce a strain which will breed true to a " throw up " of 50 per cent. " ideals " generation after generation—where the ordinary seed of commerce is incapable of a " throw up " of more than perhaps 15 per cent. This is, of course, not to say that the plant breeder will *not* have done a great deal more if he can breed a strain which will breed true to the ideal aimed at, to the extent of the full 100 per cent.

The essential point to seize upon is, however, that it must inevitably take a very long time to breed a strain which attains to reasonable genetical purity, while in many cases it need not take many generations to achieve something which breeds true within a limited range of variability and such that the seed will give rise to a population of plants which in the aggregate is vastly superior to any population of plants which can be produced by the seed of commerce as ordinarily obtainable.

The procedure which I advocate is, therefore, to start operations by adopting relatively rough and ready methods—methods which can only be expected to produce a population of plants which will give reasonably high percentages of the ideal aimed at. The plant breeder should, however, always be coming along behind himself and by the adoption of methods increasingly elaborate should aim at a product attaining ever more closely to the genetical ideal of purity.

In my view the technique to be adopted should, therefore, be manifold, and at any particular time a particular breeder working on a particular species would have material in all stages being worked up by a variety of methods—each method being appropriate to the stage at which he had arrived relative to any particular ideal.

In a broad way the methods which I am employing with cocksfoot may be grouped together in four categories. These methods must be regarded as additional and complementary to those previously described by Mr. Jenkin.

Concentration of Types.

All that is here aimed at is to produce seed which will give rise to a high percentage of easily recognisable and highly desirable " growth-form " types—types which are abundantly represented in Nature by the species in hand. To do this on a scale that may be of considerable economic importance does not by any means always necessitate in the first instance an effort to ascertain the genetical constitution of the potential parent plants. It is a matter of group breeding, and the number of mother plants used will be very considerable.

Purification of Types.

The method employed here is to group comparatively few carefully selected plants. The method is hazardous, for if the genetical behaviour of the plants grouped has not been explored, it is of course unlikely that special excellencies will be rendered true breeding by this means.

In the case of cocksfoot I none the less have obtained useful material by adopting this plan during the early years.

Strain-Building.

My own experience was that when I had worked for some years with my natural types, my eye had gained much in critical judgment, and I was able to select super-type plants ; and to isolate, breed or select material breeding true to these super-type plants now became my ideal. When one advisedly starts to do this, one must necessarily to some extent explore the genetical constitution of one's parent plants and very much tighten up one's technique. It does not follow, however, in order to bring about a degree of improvement in itself well worth while that one need necessarily hybridize, nor indeed that one need necessarily concern oneself with the modes of inheritance of the various characteristics of one's species. By adopting an appropriate technique it is often possible to find out comparatively quickly whether one can achieve what one wants, or at least go a long way towards achieving what one wants, without anything like a full knowledge of the facts. If this proves impossible then the investigator has no alternative but to conduct innumerable and detailed genetical researches at the outset.

Hybridization and Pure Lines.

In the last resort it will be necessary to proceed by such methods as these in the case of every species if it is desired to bring about the greatest improvement that is possible. The methods employed at Aberystwyth have been fully described by Mr. Jenkin—and such methods are undoubtedly the only ones applicable to certain species. They are, moreover, the only methods by which one may hope to achieve a reasonable measure of success if it is desired to breed for such special attributes as, for example, high protein content, high mineral content, absolute resistance to a particular disease, or absolute purity in any respect whatsoever.

I am myself adopting the hybridization and pure line procedure with cocksfoot as one method of strain-building and with a view to attaining to the maximum of what may be possible in respect of smoothness of leaf and in respect of maximum seed production in a pasture type.

It is necessary to emphasise that the methods most suitable to any particular species must very largely depend upon the fertilization affinities of that species and upon its ecotypical relationships. In this connection it is well to emphasise that the two great herbage Natural Orders—the grasses and the clovers—show the widest variation as between genus and genus, species and species, in these two important respects. Thus quite a number of grass and clover species, particularly the annuals, are as self-fertile and as self-vigorous as are the ordinary cereals.

These introductory remarks have seemed necessary, for the breeding of cross-fertile grasses presents an entirely different problem from the breeding of self-fertile cereals, and the point of view of the cereal breeder has very largely dominated the point of view of the genetically-trained plant breeder ; and, besides, the technique now to be explained is the outcome of the point of view which I have endeavoured to enunciate. Further than this, it is one thing to adopt what may appear to be haphazard methods because one knows no better, and quite another to adopt them of set purpose with a full appreciation of their limitations and with a full appreciation of precisely how far they may be expected to carry one.

Isolation and Protection from Stray Pollen.

Mr. Jenkin has dealt fully with the question of the selection of a suitable fabric for the purposes of isolation and with the methods employed at Aberystwyth for selfing. There remains the question of group isolation, and three methods have to be considered, namely, (1) open isolation by distance ; (2) isolation in cages ; (3) isolation in greenhouses.

Whatever method be adopted to achieve group isolation, as the result of my eleven years experience with cocksfoot, I have been driven to the opinion that the first essential to success is as far as possible only to " match-up " (= isolate together) plants which will come into flower contemporaneously. It is desirable that a " great mist " of the desired pollen should flood the stigmas of the isolated mother plants when these stigmas are receptive—desirable in fact that the " pure " pollen should be the first pollen and by far the most abundant pollen offering to the " pure " stigmas. It is evident that this matter of date of flowering is one to which the breeder must pay the closest possible attention when selecting his plants for grouping—for it is a matter upon which more than almost anything else his success will depend ; a difference of even one or two days in the zenith of flowering between the plants isolated is likely to exert a decided influence on the ability of the plants to inter-pollinate all round and on the powers of resistance of the plants to stray pollen.*

Open Isolation by Distance.

With regard to isolating out of doors without resort to any artificial protection, two points (over and above the crucial matter of date of flowering) are important. In the first place, it is necessary that the isolation area should be as large as possible, and in the second place, that the area should be situated as far as possible from other areas upon which the species grows. In this latter connection it is desirable to emphasise the fact that in the early stages of grouping, " sufficient economic purity " is altogether more important than heavy seed yield. It is only necessary to aim at " sufficiently good " seed yield to make a nucleus for further " building up". Two thoughts arise in this connection, the one that the opportunities for isolation are at their best, and indeed are perfect, in countries or districts where a particular species is not grown—and this thought opens the door to avenues to almost unlimited co-operation between the plant breeders concerned with an assemblage of habitats so unique as that afforded by the British Empire. The second thought is that within the confines of a district of limited size one may attain to " sufficiently good isolation " *cum* " sufficiently good seed crops " by growing the material to be isolated on a habitat, agriculturally and ecologically considered, quite unsuited to it. Thus, I have successfully isolated cocksfoot 8 miles from Aberystwyth on a hill sheep walk at 900 feet above sea level on a small and specially prepared area.†

Protection by Fabric Cages.

The cages employed have consisted of fabric‡ " tents " stretched over appropriate frames. In some cases the sides of the tents have been made of light canvas and only the tops covered with the standard fabric. More recently the tops of the frames have been covered with glass lights and the sides have been covered with the standard fabric. On the whole, decidedly better results have been obtained since glass roofs have been introduced. Shutters have been fixed at ground level, and these are always opened as soon as seed setting has finished.

Although as the result of Mr. Jenkin's tests it seems probable that agitation is not essential in the case of selfing, this is not by any means to suppose that it is not necessary in the case of inter-pollination. If there is no agitation the tendency in the still atmosphere of a cage is for the pollen of any particular plant to fall down direct on its own stigmas and to absolutely flood the stigmas with such pollen. The matter of agitation presents great difficulties ; in the first place, there is the risk of the introduction of stray pollen, and in the second place, the anthers should be shaken at the time of maximum dehiscence. With regard to stray pollen a slight degree of contamination would be less serious than in the case of selfing—for one thing, with a number of

* This is a matter now under detailed investigation.

† The seed crop was light, but for my purposes quite sufficient, and the genetical evidence suggested that the isolation from stray pollen was emphatically such as to give " sufficient economic purity ". The evidence will be presented in detail in another connection elsewhere.

‡ The standard fabric previously described by Mr. Jenkin (see p. 11).

plants closed in together a stray grain would not be in any favoured position, and for another thing, the genetical behaviour of the mother plants as such is not under test. Caging is usually adopted for the production of progeny plants. The method of agitation employed has been to introduce a stick on the lee side of the cage through the shutter (at ground level) and to shake the panicle-bearing stems. As far as possible the operation has been conducted at, or soon after, flowering time, i.e., about 9 a.m.

After adopting this method of panicle agitation, I found that my inter-pollination was satisfactory up to a point, and that my protection remained " economically sufficient," but I soon suspected that I but seldom, if ever, achieved inter- and reciprocal pollination as between all the plants encaged to the full extent of what was possible. My suspicions were further aroused when I took to harvesting the seed from each of the encaged plants separately ; the crop per plant varied over altogether too wide a margin, and this, together with the fact that the progeny from some of the plants often gave an excessive number of albino and virescent seedlings caused me to assume too frequent lack of cross-fertilization and too much selfing.* Part of the trouble, as before explained, is undoubtedly due to the difficulty in matching-up the plants so that they do in fact come into flower contemporaneously. I have evidence which shows that it does not follow that because in a previous year plants A and B reached their zenith of flowering on the same day, growing unhampered out of doors, they will necessarily do so the next year in a cage. As Mr. Jenkin, however, has shown, it is not only a matter of flowering date, for plants which are relatively highly self-fertile may be very largely selfed even when encaged with other plants presumably flowering at the correct time. For the purpose of reciprocal inter-crossing it would, therefore, seem desirable only to encage plants which have but a poor capacity for self-fertility. Even in the case of cocksfoot (relatively self-fertile as is the species as a whole) plants with very high self-fertility are not nearly as common as those with low self-fertility. On this account it would seem desirable to introduce as many parent plants as possible into each cage—for one highly self-fertile plant would then be responsible for much less harm than if caged with but two or three. While from the point of view of the time factor in relation to inter-pollination, the larger the number of plants the better are the chances of cross pollination.† The number can of course be increased by breaking the plants down (I usually break down to four)—and putting together in a cage say 10 or 12 small plants rather than 3 or 4 large ones. By this means the number of mother plants per cage can be increased and the chances of inter-pollination between the plants as a whole by that much increased. By duplicating the groupings in separate cages considerable supplies of seed can of course be obtained.

I have now totally abandoned the use of outdoor cages, and instead use greenhouses. The cage, however, has undoubted applicability provided its limitations are sufficiently realized. At Aberystwyth it is only in exceptionally fine years that the cages provide good conditions for the setting and ripening of seed, while it is exceedingly difficult to make such cages storm-proof. It remains a fact, however, that some of the most promising of my current material has been built up by the cage method.

Protection by Greenhouses.

The objection to cages, discussed above, at an early stage suggested the advisability of using greenhouses, while the feasibility of using greenhouses was suggested by the fact that all the critical genetical hybridization work of the Station had from the outset been conducted in greenhouses.

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* I am not of course assuming that the presence of albino and virescent seedlings as such necessarily proves that the plant producing them was not cross-pollinated—for indeed such an assumption would not be justified by the facts—but in cases other than where one may be advisedly breeding to produce albino and virescent seedlings the large amount of material which I have handled leads me to regard it as a good " rule " in connection with the methods of economic breeding, such as I am discussing, to suspect selfing when such seedlings occur in appreciable quantity.

† It must be borne in mind that even if a particular highly self-fertile plant does not show incompatibility with the pollen of other plants, it may yet be that the pollen from some plants may be more effective in preventing selfing than that from others. This is a matter of supreme importance and one that we have not yet been able to subject to detailed investigation. If, however, highly self-fertile plants reacted differentially to the pollen of other plants in relation to their reaction to their own pollen—then we should have another reason for encaging together as many plants as possible.

Let me insist that I am only asking my greenhouse to afford " economically sufficient " isolation from stray pollen, and such evidence as is at present available indicates that with reasonable precautions an ordinary cheap greenhouse is competent to do so* (Plate I, fig. 1). Consider the stillness of the atmosphere within a completely closed greenhouse. Consider the water film between the panes of glass in a house full of actively transpiring plants.

Certain precautions have to be taken. The house must be shut well before flowering time and kept shut as long as the stigmas are receptive. The panicles must be shaken. In order to shake the panicles Mr. Watkins (the Superintendent at the Farm) devised the scheme of passing binder twine round the panicles of each plant and bringing one end of the twine through a small hole to the outside of the house : by pulling at the twine the panicles can be abundantly agitated.

Our experience at Aberystwyth in short is to the effect that by resort to the use of greenhouses we can obtain " sufficiently good " seed crops of " sufficiently protected " seed for the standards of purity at which we are aiming in connection with a large proportion of our breeding work. The greenhouse has a further great advantage over the cage in that it allows of the bringing together of a much larger number of plants, the importance of which will shortly be made apparent. While if it is desired to isolate but a few plants together, a fabric cage can be employed within a greenhouse and the cage can be removed as soon as flowering is over, leaving the developing seed under reasonably favourable conditions and safe from the effects of wind or the ravages of birds.†

With these remarks on the general question of isolation and protection I will pass to a more detailed consideration of the various methods of breeding that have been outlined.

Concentration of Types.

To achieve this, I have in effect adopted the well-known method of mass-selection, but with certain modifications. I have always aimed at obtaining my type plants from one and the same habitat—and from a habitat where the type which I am desiring to concentrate already existed in a highly concentrated form—already existed if possible as the only type peopling a certain habitat (i.e., as a definite ecotype), or failing this, as the chief type contributing to the population of plants on a certain habitat : my underlying idea of course being that auto-selection would have gone a long way, and that I should be dealing at the outset with a relatively pure population.

It has, however, to be borne in mind that the vigorous type plants one is desiring to " concentrate " are likely to be anything but completely homozygous—while it is perhaps the most vigorous plants that are the most heterozygous. Even when a particular habitat is peopled by plants which appear to be remarkably uniform, it cannot be taken for granted that such plants have attained to anything approaching to complete homozygosity, for the growth form in question.

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* I have three large families which were isolated in greenhouses in 1929 now standing in plant rows—the standard of purity of each is obviously excellent, but a critical examination of the material cannot be made until heading stage this season (1931).

† At the Welsh Plant Breeding Station we use houses which are completely separated and employ two standard sizes, i.e., 20ft. by 10ft. and 30ft. by 12ft.—at present we have available 19 houses of the former size and 11 of the latter, and also a house 100ft. by 12ft. which can be divided into partitions or in which fabric cages can be placed. In all cases the plants are planted *in situ*—the greenhouses being in effect glazed beds. It is possible that as time goes on, if the houses are continually used year after year for one species, the soil may become contaminated with the spores of some disease (*Epichloe typhina* in the case of cocksfoot) or for other reasons become unhealthy. This is a danger to be kept in mind and one which could presumably be obviated by renewing the soil periodically, or by methods of soil sterilization, or by resort to pots. Any one of these methods would be troublesome and expensive, and of course need not be contemplated until the necessity arises. I may add that we were not equipped with greenhouses until 1929, and consequently we have not yet been able to work out a complete technique for each species in respect of such matters as the best distance apart for planting, the best scheme of manuring to employ, the amount of watering and particularly during the period prior to shutting the house up—for of course no watering is possible during pollen shedding. It is worth noting that during the autumn and after the seed has been harvested and the standing plants removed, our greenhouse space is proving invaluable in connection with conditioning seed that may have been harvested under poor conditions out-of-doors.

It may well be that types unsuited to the habitat, although perhaps developed as seedlings, only to a slight extent grow into adult plants. On this account and in order to ensure the absolute maximum of cross-pollination between the plants grouped, I have always aimed at isolating the largest possible number of plants together. While if a particular habitat race with which I may be dealing has as a matter of fact reduced itself to a relatively true-breeding type, the large number of plants grouped is as such no disadvantage and by tending to ensure cross-pollination reacts in the interests of both vigour and seed production within the type.

When I have been in a position to collect and select as many as about 200 type plants from a particular habitat (e.g., from the Leicestershire fatting pastures) I have isolated such plants in an isolation plot out-of-doors. In order to mix the plants up as thoroughly as possible each individual plant is broken down to four—and the mother plants are by this means placed in varying juxtaposition *inter se.*

These preliminary isolations I am now able to conduct in greenhouses, and am able to deal with upwards of 400 plants if I discard a large portion of each selected plant and actually plant out small units ($\frac{1}{4}$th to $\frac{1}{8}$th according to size of the plant) to represent each mother plant.

In the early years, in cases where only a dozen or twenty type plants were available for grouping, I had to use cages, when I arranged for as much mixing as possible by breaking down the plants and duplicating the cages.

I have also adopted a combination of the two methods—that is to say, I have started by caging and have then grown about 500 of the progeny from the caging. I have re-selected from these to make an isolation island.

These advisedly simple and rough and ready methods have enabled me to produce what I may describe as aggregate strains, which breed remarkably true within a prescribed circle of variation from generation to generation. My strain 1163 which has been largely tested under sward conditions and has given excellent results in seeds mixtures has been built up by this means. And although the early isolations were far from complete, and although the strain has now passed through four generations (out-door isolation throughout except for the first caging) it is giving a population of plants consisting of about 75 per cent. of what I regard as " improved " growth-form types, and this, coupled with very satisfactory seed production.

Purification of Types.

Little need here be said as to technique, as it is essentially the same as that adopted for concentration. Of necessity, since the number of plants selected is, in the first instance, but few (I have usually used 6 or 8 such plants), one had to start by caging, and in the case of some families I have re-selected and re-caged for some generations. Although I have achieved some very promising and surprisingly pure breeding material by this method, it is now entirely superseded by my method of strain-building. As I have said, I am not enamoured of the cage because of the uncertainty involved relative to free reciprocal pollination all round, and for another important reason—I am doubtful on account of the unavoidable limitation of space and uncertainty as to complete inter-pollination if caging permits of the use of a sufficiency of effective parent plants to ensure the continuance *sine die* of undiminished vigour generation after generation—in other words, to permit of the sufficiency of " out-breeding " in the haphazard pollinations that will take place amongst the grandchildren and great-great-grandchildren of the first inter-reciprocal crossings and to reduce to reasonable proportions the chances of matings between brother and sister plants. I have no exact or *ad hoc* evidence to guide me on this important point, but my impression, based on the behaviour of my material in general, leads me to the view that the larger the number of parent plants that in reason can be brought to enter into the original grouping (without drastically interfering with the standard of purity aimed at) the better.*

* This is a point also discussed by Mr. Jenkin, and one which raises genetical issues of far-reaching importance ; it is one, however, which it would be difficult to subject to critical experimentation and at the same time to keep within manageable dimensions. Obviously the genetical constitution of the actual plants employed would exert a profound influence, that much is certain, because in the case of cocksfoot itself, for example, I have pure lines in L_4 which are quite reasonably vigorous, although not representing the types that I want. I have also had pure lines which have died out completely in L_2. A complete genetical exploration of one's potential parent plant is, however, a long process, and I am here discussing methods of technique which shall be at once easy, quick, and economically effective.

Strain-Building.

The plan here outlined represents a half-way measure between my " type concentration " and Mr. Jenkin's " strain-building " ; the former method completely ignoring the genetical make-up of the very large number of mother plants used, and the latter method subjecting the comparatively few mother plants to genetical investigation.

In the case of any particular type I endeavour to select not less than 30 unrelated plants, all of which I regard as quintessential as to the desired growth-form and which are free from disease, free from browning and " tip burn," and are bulky. Each of these plants is then selfed and the progeny from each subsequently set out in plant row families—for my purpose I find 50 plants per family a sufficiently good guide, but in order to obtain sufficient material, if I have only a few families I usually put out about 100—200 plants per family. Those families which breed sufficiently true to type are retained (latterly working with types of which I have considerable acquaintance I base my first selfings on an expectation of at least 50 per cent. retainables), and those which show segregation from type are rejected. From the retained families I select the quintessential plants, keeping in mind also the desirability of the finally selected plants coming into flower as nearly contemporaneously as possible. I am now able, however, to allow myself a certain amount of latitude, as I shall have a number of plants representing each original mother plant, and consequently my chances of inter-cross-pollination amongst all the families is by that much enhanced—for there is most usually a certain range of flowering date within an otherwise reasonably pure family.

The number of families retained will vary considerably according to the material I am dealing with, but usually I have little difficulty in selecting about 200 quintessential progeny plants. These are then isolated together in a greenhouse, or if I have a large number of original plants giving rise to a large number of good families I may be able to select upwards of 600 quintessentials and set up an outdoor isolation island. When this is possible, I, however, also divide 200 or so of the very best plants which have come from the purest families and isolate these selected quintessentials in a greenhouse.

Such in broad outline is my method of strain-building which has involved no hand hybridization and no critical genetical researches, and for that matter no very elaborate system of note taking or of recording relative to the parent plants and their selfed families. It is a plan which is amenable to many modifications—thus, in order to ensure greater purity and greater certainty, the selection for final grouping can be deferred until a later generation, that is to say, the plants of the selected families from the first selfing can themselves be selfed. And ultimately, if one so desires, one can go back to such of the original parents as under some generations of selfing have proved themselves to be the most pure genetically, and isolate these original mother plants themselves rather than their progeny. On the whole I prefer to use the progeny plants, because it is thus possible to deal with larger numbers and therefore to add to the chances of inter-cross-fertilization as between family and family, which in the case of true-breeding material comes to the same thing as inter-cross-fertilization as between the original mother plants.

When proceeding by the methods of strain-building here outlined it is not necessary to confine oneself to plants derived from one and the same eco-habitat. The method adopted aims to have tested at least partially the potentialities of the selected mother plants, and it is type *qua* type that now alone matters. Indeed some of my best material which is being built up by this method is based on progeny grouping from mother plants obtained in the first instance in part from New Zealand, in part from Ireland, and in part from several counties in Britain.

Hybridization and Pure Lines.

The technique of strain-building based on hybridization and extensive genetical studies has been fully dealt with by Mr. Jenkin. Such methods are, of course, entirely applicable to cocksfoot, and when improvement has been carried some way by the easier and quicker means here described, the most promising material will be used for critical hybridization work.

Evidence suggests that in the case of cocksfoot it may be possible to build up satisfactory single-plant lines—and that at all events this possibility is worth exploring thoroughly. I have about half a dozen lines now in the sixth generation from the selfing of the original mother

plants which are exceptionally self-fertile. One of these gives rise to families the majority of the plants of which are completely self-fertile. These particular lines do not, however, represent types of pronounced agricultural usefulness. On the other hand, I have lines now in the fifth generation from selfing (without back- or brother and sister crossing) which are maintaining vegetative vigour and trueness to growth-form type to a marked degree, and which represent types which I regard as of supreme agricultural value. Up to the present, however, I have not been able to isolate a single line (built up from repeated selfings) having both an outstanding capacity for self-fertility and for vegetative vigour which also represents, on the basis of my standards, a first-class pasture- or a first-class hay form.

The fact that in cocksfoot it is possible to select plants which can be selfed to a very high standard of growth-form purity and which will retain at least average self-fertility is of considerable importance. It is a fact which of course opens the way to the possibility of adopting various methods of breeding based on a critical examination of the genetical make-up of the " pure lines " which can be both isolated and retained probably for an unlimited number of generations.

The possibility of being able to breed pure lines (in a manner analogous to that adopted in cereal breeding) in the case of cocksfoot, and presumably of some of the other normally cross-fertile herbage grasses, should not be ignored. The difficulties connected with isolation when seed production on a commercial scale comes to be undertaken must not, however, be underrated. For supposing a " pure line " having exceptional qualities as a herbage plant to have been established as the result of long-continued researches, there would be little hope of maintaining it in its exceptional state of purity and of excellence* unless it was even more responsive to its own pollen than to that of plants representing other and unrelated lines.

CONCLUDING REMARKS.

In Science as in Art, a technique in the last resort should be judged by one standard only—is it successful ? That is to say, in pure science does it or does not it lead to the collection of data elucidatory of one's problem ?

In applied science it is still necessary to acquire data elucidatory of all aspects of one's problem, but it is eminently desirable to do more than this. If a problem is in fact of real economic concern it is of the first importance to find a solution, or at least a partial solution, as quickly and as cheaply as possible.

In the realm of herbage breeding by no means the easiest part of the problem is to ascertain with reasonable assurance precisely to what ends it is desirable to breed. The actual breeding work must inevitably entail detailed researches—must inevitably demand considerable resources, and to be carried to a successful issue must take no mean period of time.

Before critical breeding work on any particular term of reference is embarked upon, it is therefore very desirable to have fully explored the agrostological potentialities of the species taken in hand. This of course to ensure that one is really trying to breed in sympathy with an urgent economic need. It was primarily with a view to this end that I started my " breeding " work on cocksfoot by the adoption of rough and ready methods. The aggregate strains I early acquired proved invaluable for sward trials and yielded important information as to the trend of sward behaviour of sharply contrasting types : information in fact which has proved its worth in relation to the whole problem of herbage breeding.

When assembling material in order to begin studies on any particular species it is a relatively simple matter to organise a certain amount of group breeding. The seed so obtained will in any event be essential for preliminary sward trials. While if some of the material so " bred " proves to be relatively true to a desirable type, it may be well worth growing it on and actually marketing it as a stop-gap product, pending the production of a greatly improved strain of reasonable genetical purity.

Since in the case of nearly all herbage species improvement by breeding has been practically untried the field is an exceptionally wide one. In many instances it follows, therefore, that important improvements are likely in the first instance to be possible by the adoption of

* It is perhaps hardly worth while to produce the purest of gold if it is bound to become alloyed, while to subject an alloy to the liability of a little further contamination is relatively unimportant.

comparatively simple methods. Ultimately, critical methods, such as are already abundantly called for in the case of the more generally used grasses and clovers, will have to be applied to all species having real economic significance.

In conclusion, it does seem desirable to emphasise the necessity of basing critical genetical breeding work on a solid foundation built of well-planned investigations on the grasslands to be catered for and the relation of one's species to these grasslands. In this connection it is worthy of remark that all of us who are concerned with herbage breeding at Aberystwyth have served a considerable apprenticeship to " general grassland investigations ".

It is my firm belief that in the realm of herbage improvement by breeding we shall only reap the full harvest of what is undoubtedly possible by the heartiest co-operation of the ecologist and the geneticist.

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METHODS AND TECHNIQUE OF BREEDING RED CLOVER, WHITE CLOVER, AND LUCERNE.

BY

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INTRODUCTION.

Since the rediscovery of Mendel's laws of heredity our knowledge concerning the genetics and methods of breeding of cereals and a number of other self-fertile crops has made very rapid strides, but unfortunately the state of our knowledge with regard to many of the other agricultural crops is in a far less satisfactory position. Until fairly recently very little attention had been devoted to the improvement of the herbage plants, mainly owing to certain inherent difficulties connected with their modes of fertilization, and, with a few notable exceptions, no attempt was made to place the breeding of grasses and clovers on a scientific basis.

The aim of this article is to describe the methods and technique employed at the Welsh Plant Breeding Station for the breeding of three leguminous crops—red clover, white clover, and lucerne. The breeding of red clover was begun in 1921, but the work on the other two crops was not initiated until three years later. As the methods adopted for all three crops are somewhat similar, to avoid unnecessary reiteration only those employed in the improvement of red clover will be discussed at length, while but brief reference will be made to the main points in which the methods utilized in the case of the other two crops differ from those described in detail.

On looking through the literature on the subject it soon became evident that apart from a few more or less successful efforts at mass selection very little attempt has been made to improve these crops on systematic lines. Though a considerable amount of work has been devoted to the modes of their fertilization, the results obtained by the different workers were often so contradictory as to necessitate repetition of many of the investigations.

The scheme of breeding that will be described has been gradually evolved as a result of 10 years continuous work. As might be expected from the fact that the scheme had to be built up *ab initio*, several mistakes were made during the earlier years, many of which proved costly in time, money and material.

This paper is written in the earnest hope that it may be of some assistance to other breeders of these crops by enabling them to avoid at least some of the pitfalls.

RED CLOVER.

Self-Fertilization.

It is obvious that thorough knowledge of the mode of reproduction of a crop is essential before a workable scheme of breeding can be devised. The mode of fertilization of red clover has been subjected from time to time to a large number of investigations, the results of which have been summarized by the writer in a previous publication (Williams, 1925). Although different workers hold divergent views as to the exact degree of its self-sterility, there is a general consensus of opinion that red clover is mainly self-sterile—a conclusion which is amply supported by the figures below summarizing the results obtained from artificial self-pollination at Aberystwyth up to 1929.

It is evident from these figures that although quite an appreciable number of the plants are capable of producing a few seeds—only one or two in most cases—on being artificially self-pollinated, the crop as a whole is practically self-sterile. A few plants,

Year.	Number of plants self-pollinated.	Number of plants which produced seeds.	Total number of florets* self-pollinated.	Total number of seeds.	Percentage number of seeds.
1921	16	4	1540	52	3.45
1922	22	11	6183	59	0.95
1924	24	3	2528	8	0.32
1925	8	1	1191	2	0.17
1926	17	1	4705	4	0.09
1928	76	1	9381	65	0.69
1929	99	4	9647	110	1.14
Total ..	262	25	35175	300	0.85

* All these florets were selfed by hand. The percentage self-fertility was appreciably less when the florets were selfed by merely rolling protected heads between the fingers.

however, have been isolated which are more or less self-fertile. One F_1 plant, viz., 344(1)263, which is the most highly self-fertile red clover plant found so far, has produced respectively in three successive seasons 75, 30 and 42 seeds per 100 florets artificially selfed. The progenies of the highly self-fertile plants vary widely in regard to their degree of self-fertility; some of the plants are completely self-sterile, while others are as self-fertile as, and in some cases appreciably more self-fertile than, the mother plants. There is no doubt from the investigations of Fergus (1922), Kirk (1925) and Sylvén (1929), that self-fertility in red clover is an inheritable character, as all three workers have succeeded by repeated selection in isolating fairly highly self-fertile lines of red clover.

From comparison of results obtained by different investigators it would seem that red clover varies appreciably in different regions in regard to its degree of self-fertility. The evidence available indicates that it is distinctly more self-fertile in California in U.S.A. (Fergus, 1922), in Ontario in Canada (Kirk, 1925), at Svalöf in Sweden (Sylvén, 1929), than at Øtoftegaard in Denmark (Frandsen, 1916), at Hohenheim in Germany (Schlecht, 1921), and at Aberystwyth. That this difference is not a varietal character is shown by the fact that at Aberystwyth there has been no apparent difference between the various varieties—including American, Canadian and Swedish—so far tested in respect to their degree of self-fertility.

Though it is admittedly possible, granted an unlimited amount of time and patience, to isolate at least fairly self-fertile lines of red clover, contrary to the view held by a number of other workers the writer is firmly of the opinion that the production of improved strains is not possible by self-fertilization. This conviction is based on the following grounds: firstly, in any population of red clover the proportion of plants of outstanding merit is very small, probably not more than 1 to 2 per cent.: even under the most favourable conditions red clover is so extremely rarely self-fertile that the odds against these few promising plants being autogamous are very great; secondly, assuming that by some lucky chance the best plants were to prove self-fertile, in red clover the loss of vegetative vigour resulting from inbreeding is so pronounced that the lines would in the vast majority of cases be totally useless for all practical purposes until they were out-crossed.

In this country red clover is cross-pollinated almost entirely by means of certain species of humble bees (*Bombus*) with the assistance of honey bees in some seasons. Although butterflies, moths, and other insects may sometimes be found visiting the flowers, they are unable to effect pollination.

STUDY OF EXISTING VARIETIES.

One of the prerequisites to successful breeding is that the breeder should have an intimate knowledge of the crop he is working on. During the first six years (1919—1925) a series of investigations were undertaken with the object of obtaining more information than was then available concerning the botanical and agronomic characteristics of the numerous varieties, forms and nationalities which constitute red clover. The first step was to determine by means of extensive series of field trials their adaptability to the local conditions and their suitability for different purposes. Samples of seeds representing practically all the main forms were

obtained from different countries and sown not only on the Plant Breeding Farm but also at various centres in the counties. The seeds were sown alone or in simple mixtures. In order to test the persistency of the different varieties the trials were continued for at least two harvest years, while some were taken into the fourth year. With the exception of a few trials designed to test the behaviour of the different varieties under grazing conditions which were cut every four to five weeks throughout the growing season, all the trials conducted on the Station's experimental grounds were mown twice as hay and aftermath each year. The standard size of the plots generally used at the Station was one-hundredth of an acre replicated four times, but in the counties duplicated one-tenth acre plots were preferred. Field trials with commercial samples have now been discontinued, but small-scale trials are still being carried out in which all samples received are sown in duplicated ten-foot drills. Whenever possible these are kept under observation for at least two years. It is interesting to note that these small drill trials have been the means of bringing to light several lots of outstanding merit.

As the results of these investigations have been fully reported in one of the Station bulletins (Williams, 1927), it suffices to state that a considerable amount of valuable data was obtained concerning the agronomic properties of the different varieties,—their yielding capacity, permanence, winter-hardiness, disease resistance, ability to stand grazing conditions, seasonal growth, and other characteristics—information which has since proved to be of great value not only to the farmer, but also to the plant breeder. Speaking generally, of the late varieties the best results were given by two local strains, Montgomery and Cornish Marl, while the heaviest yielding variety of the early clovers was another local strain, grown in the Vale of Clwyd in North Wales, and it is from these local varieties that the most promising breeding material has been obtained.

Red clover is an exceedingly variable species ; it consists of a large number of more or less distinct varieties and races, in fact practically every country in which red clover has been cultivated for some time has its own characteristic forms. In the case of countries such as America, and New Zealand and Tasmania, in which red clover is not indigenous, it is evident that the distinct races now found in these countries have become differentiated from the European races from which they originated entirely as a result of natural selection. But in the case of the local late flowering races of North Europe and Asia, it is highly probable that inter-crossing with the local indigenous wild races has also played an important part in their formation.

STUDY OF VARIATION WITHIN THE VARIETIES.

Owing to the fact that most of the plants within a variety have certain major characteristics in common, the different varieties appear at first sight to be fairly uniform. If, however, a number of individual plants of any variety be closely examined it will at once become evident that a variety is nothing less than a heterogeneous collection of an extremely large number of distinct types ; in fact, the range of variation within the variety is so great that it is practically impossible to find two plants exactly alike in all respects. From the same variety it is possible to select types differing not only in numerous morphological characteristics but also in a great many important agricultural traits such as yielding capacity, early and late growth, winter-hardiness, permanence, earliness of flowering, stem and leaf formation, and disease resistance. Nearly every variety, in fact, contains such a wealth of material as to offer ample scope for production of greatly improved forms. If red clover were self-fertile it would be a comparatively easy matter to isolate types which would be far superior to the existing varieties, but since it is almost exclusively cross-fertile the breeding of this species presents many difficulties which render the work long and arduous.

Before starting to breed it was considered advisable to make a detailed study of a number of individual plants of those varieties which were most likely to give the best breeding material. For this purpose, in 1922, some 30,000 seedlings of Montgomery, Cornish Marl, English late, Swedish late, American Mammoth, English broad red, Vale of Clwyd, American medium and wilds from various sources were planted out* in lines 27 inches apart, and with 24 inches between

* The method of planting employed at this Station is described on p. 59, while the procedure adopted for note taking is dealt with on p. 60.

the plants in the lines. During the three subsequent years critical studies were made on the individual plants, and data were collected on characteristics such as growth habit, time of flowering, size of leaves, number and length of stems, number of internodes, susceptibility to diseases, rate of mortality, winter-hardiness, and seasonal growth. The results of some of these investigations have already been reported (Williams, 1927).

SELECTION OF PARENT PLANTS.

As may be expected on *a priori* grounds, the native varieties—Montgomery, Cornish Marl, English late, Vale of Clwyd, broad red and wild reds—from which most of the parents are now selected, have so far given the most promising breeding material. A number of the selections from some of the local strains of Swedish late have also given encouraging results, but those from all the other foreign reds from which selections have been made have proved disappointing.

Some of the methods employed in selecting parent plants and the sources from which they are obtained are briefly described below.

(1) A large number of seedlings of the commercial samples are planted specially for this purpose. To increase the chances of obtaining plants of outstanding merit, several thousands of plants of the best varieties are planted. The seedlings are put out serially in lines of ten each—an arrangement which greatly simplifies and reduces the work connected with labelling, preparation of the field note books and recording.

The number of years during which these plants are kept under observation depends on the aims which the breeder has in mind. If the object is to produce improved strains for short duration leys, the selections are made at the end of the second year, if on the other hand the chief aim is to breed persistent strains for long duration leys, the plants are not selected until the end of the third or fourth year. Apart from recording the plants that have died back as a result of planting, the only other notes considered necessary in the planting year are a few general observations on each population as a whole, but during subsequent years careful observations are made three times each season—about the end of April, at the end of June, and again at the end of September—when marks* ranging from 1 to 5 are allocated to each plant representing its economic value as indicated by its relative size, leafiness, freedom from disease, and other qualities of agronomic importance, and entered in field note books. As it is absolutely essential when selecting parent plants for crossing to take into consideration their general performance throughout the whole of their life histories, a scheme of marking such as that described above is indispensable. As already stated, in all cases in which the final object is to breed new strains with greater permanence, the parents are not selected until the third or fourth year in order to allow the short-lived types which are present even in the most persistent varieties to die back. This procedure greatly simplifies the work of selection. To give only one example : a population of 1,195 plants of English late was reduced by winter-killing and other causes to 920 plants by the spring of the second year, to 597 plants by the third year, and to 161 plants by the fourth year, when the selections were made. The marks showed that of the 161 surviving plants only 35 had given uniformly good results throughout the whole period, and of these only 18 were finally selected for breeding, the other 17 plants being discarded on account of extreme hairiness and other undesirable morphological characters.

(2) As will be explained later, a large number of plants of some of the best late and early varieties are put out with the pedigree material every year for the purpose of comparison. These " control " plants usually afford a regular supply of new parents for economic breeding. The method of selection is similar to that described above.

(3) Promising breeding material has also been obtained from old fields and trial plots. In this case it is important to discriminate between genuine old stands and those which have been produced wholly or in part as a result of self-seeding. Fields which have been harvested for seed or which have been so badly managed that the plants have been allowed to run to seed invariably contain a large proportion of relatively young plants. As the writer has found

* The system of allocating these marks is described on p. 60.

to his cost, it is mere waste of time to make any selections in such fields. For instance, on one occasion some 300 plants were dug up from a reputed 10-year-old field in Montgomeryshire, and potted. In due course the best plants—about 100 in all—were inter-crossed. With the exception of one family, which has proved exceptionally persistent, all the progenies of these supposed 10-year-old plants were most disappointing as regards their permanence. It transpired later that this field had been harvested for seed four times during the ten years, and it is highly probable that the vast majority of the plants present were of comparatively recent origin.

Old fields which have been mown for hay or closely grazed every year since their inception are fairly reliable sources of genuinely persistent types, but the most trustworthy sources of all are trial plots which have been cut for hay and aftermath every year since they were laid down, for under these conditions the plants have no opportunity of self-seeding.

The selection of plants for breeding purposes from old fields is usually made in the autumn, and as it is not possible to evaluate with any accuracy the agronomic properties of the plants in the fields, many more plants are potted than are actually required. These are kept under close observation during the following spring and early summer, when they are again selected before they are paired off for inter-crossing.

(4) A method of selecting for winter-hardiness which is sometimes employed is to leave the plants that had been used for crossing during the preceding summer in the open in pots throughout the winter. As the soil in the pots is often completely frozen for several days, it is evident that the plants exposed in this manner are subjected to a much more severe test of winter-hardiness than if they were in the field ; it was found that as a rule only from 5 to 10 per cent. were able to persist under these conditions. The surviving plants were inter-crossed in pairs. As shown below, the results obtained were exceedingly variable, and while the progenies of some of these crosses gave high persistent values, others were nearly as short-lived as the original variety from which they were derived.

		Percentage number of plants alive.		
Year planted.		2nd year.	3rd year.	4th year.
1926	Original variety	96	49	6
,,	F_1 229 (1)	93	60	19
,,	F_1 230 (1)	100	91	62
1928	Original variety	92	8	—
,,	F_1 484 (1)	81	15	—
,,	F_1 485 (1)	98	51	—

This method of selecting for winter-hardiness, though it has proved fairly effective, entails such a heavy outlay in pots, which become so badly cracked after they have been left in the open through the winter, that it cannot be recommended for general use. Probably a better method would be to subject the plants to low temperatures in a refrigerating apparatus specially designed for the purpose. Such an apparatus has been devised and used by Dr. H. N. Frandsen, Director of the Experiment Station at Øtoftegaard, Denmark, with most encouraging results.

VEGETATIVE PROPAGATION.

During the first three years—1921 to 1923—all the hand pollinations were carried out in the field on plants *in situ*. For reasons to be discussed later, this method proved so utterly unsatisfactory that it was discontinued. Since 1924 practically all the hand crossing has been done on potted plants under glass. During the first two years following the adoption of the latter method the plants were dug up and potted *in toto*, and this was done either in the autumn or spring. The autumn pottings were not very successful as a large number of the plants, despite the fact that they were kept under glass during the winter, were unable to take root, and perished before the spring. As regards the spring pottings, the best results were obtained by transplanting within a week or two after active growth had started. The casualties were usually very heavy when the plants were disturbed too early in the spring, while on the other hand if the plants

had made an appreciable amount of growth before they were potted, they were generally weak and often succumbed to anthracnose and mildew. On the whole this method was quite successful, provided the plants were moved at the proper time.

In 1925 an attempt was made to propagate red clover vegetatively by means of cuttings. The method proved so consistently successful that it is now the only one used in dealing with the plants selected for practical breeding, while many of the genetical crosses are made on similar material. Provided ordinary care is taken in choosing suitable shoots for the purpose, red clover can be propagated by cuttings almost as readily as white clover—if the original plants are strong and healthy the number of casualties is seldom more than 1 to 2 per cent. Young basal shoots with a large number of nodes constitute the best material, as they take root and form new crowns much more readily than cuttings of stems with extended internodes.

The cuttings are always taken in October soon after the final selections have been made for crossing in the following year. They are then planted—one to each pot— in small 4-inch pots, and kept in a cool greenhouse over the winter. The soil used consists of 1 part sand and 2 parts good light loam, which has been sterilized to keep down weeds, and to this a little phosphatic manure is added. Though for many purposes one cutting per plant is sufficient, as will be seen later when discussing methods of breeding, it is sometimes necessary to take several cuttings—in some cases as many as ten—from some of the most promising plants.

Early in March the cuttings are transplanted from the small pots to large 10-inch pots, and after they have been in the glasshouse for a few more days they are placed in the open and left there until they are required for pollination. (Plate II, fig. 5).

When left in the open, the majority soon develop into strong healthy plants, which in the course of time produce a profusion of flower heads. Though they are generally far less susceptible to anthracnose than in the field, they are much more readily attacked by mildew, particularly when kept under glass, but, fortunately, this disease can be fairly easily combated by using appropriate sprays. There is, however, no known remedy for a troublesome and puzzling disease, which for want of a better term may be called " yellowing off " ; its exact nature has not yet been thoroughly investigated. It is frequently present on spaced plants on the experimental grounds, but appears to be much more prevalent on plants in pots. It is very rarely seen on seedlings and generally makes its first appearance on the plants in May, when it may be recognised by the slightly yellow tinge of the leaves ; but as the plants develop they become first distinctly yellowish green, and later when in bloom dull reddish green in colour. The affected plants generally produce some flower heads, but have never yet been found to set seed.

CROSS-POLLINATION.

As already stated, during the first three years cross-pollination was done almost entirely in the field. During the first summer (1921) which was exceptionally dry, warm and sunny—conditions highly favourable for seed setting—the results were most encouraging, hand pollinated flowers yielding 63 per cent. seeds ; but on account of the unfavourable conditions which prevailed during the pollination seasons the results obtained in the two subsequent years were not nearly so satisfactory, the average fertility of the few hand crosses that could be effected during these seasons being 42 per cent. and 33 per cent. respectively. The main flowering period of red clover is July and August, and at Aberystwyth July is usually the wettest month of the summer, while August, though not usually as wet as July, is far from being one of the driest months.

Even under the best conditions the hand-pollination of a large number of plants such as is necessary in economic breeding is often a long and laborious operation, but when the work has to be performed in the field the difficulties are very greatly increased, as the breeder is completely at the mercy of the weather. For instance, in the period covered by the last 10 years (1921—1930) only during two years, viz., 1921 and 1925, was it possible to cross red clover in the field with a fair measure of success ; all the other seasons were for the most part much too wet and cold. In wet weather it is often impossible to do any crossing for days on end, and even if it were possible it would be mere waste of time, because the pollen becomes non-viable and bursts almost as soon as it comes into contact with water. The few crosses that are effected are damaged by rain and storms, while anthracnose, which is rampant in wet seasons, also takes a heavy toll. The loss which a wet season entails would not be so serious if it were possible

to repeat the same crosses next season, but unfortunately a considerable number of the plants are invariably killed off during the following winter. A great deal of valuable material has been irretrievably lost in this way.

As already explained, since 1924, nearly all the hand-crossing has been done on plants in pots under glass. The dimensions of the pollination houses are : length—25 feet, width—10 feet, height to eaves—5 feet, with two movable benches—one on each side—18 inches high and 3 feet wide. For pollination work the glasshouses have a great many advantages over the field. To enumerate only a few : under glass the work can be carried on irrespective of weather conditions, and with proper organisation the pollination season can be extended from June to October with the result that a very considerably larger number of crosses can be effected ; also as indicated by the figures given below the fertility of the crosses is appreciably higher, while the seeds are of very much better quality.

	Percentage fertility.	
Year.	Field.	Under glass.
1922	41.8	—
1923	32.6	—
1925	—	72.7
1926	27.4	44.6
1927	13.6	28.8*
1928	18.3	51.8
1929	31.3	41.3
1930	—	50.1

Two methods of cross-pollination employed are described below.

Hand Pollination.

Before the plants are brought in, the glasshouses are rendered bee-proof by covering from the inside all the windows and skylights with wire gauze. As an additional precaution against bees the doors are protected by wire gauze porches so that each house has a double door. A few days before the plants start flowering they are paired off into their appropriate crosses, and after they have been allocated temporary cross numbers they are taken into the glasshouses when their morphological and other characters are scored and entered in the "study book", and the necessary particulars are recorded in the " pollination book ".

As already mentioned, red clover plants when under glass are particularly susceptible to mildew, and plants which are normally completely free from the disease in the field are often very badly infested in the greenhouses. A bad attack of mildew has the effect of materially reducing the fertility, and in some cases it actually kills off the plants. When the plants are brought in they are at once sprayed against mildew with a commercial preparation containing potassium sulphide. This is very effective in holding the disease in check for 7 to 10 days, when it starts spreading again, but as the preparation is apt to burn the flowers, the plants cannot be sprayed again if they have started to bloom.† Though heavy spraying with water assists in preventing the disease from spreading rapidly, it is advisable to place the plants in the open as soon as possible after pollination. It is interesting to note in this connection that the first year plants are not affected by mildew to nearly the same extent as mature plants ; they may therefore be kept in the glasshouse until the seeds are ripe without any risk of impairing their fertility.

Again aphides, which are much more partial to red clover than any of the other crops with which the writer is concerned, cause even more damage than mildew, unless they are kept under control. They can be very effectively controlled by fumigating with nicotine. This is done every two weeks throughout the pollination season, as it has been found to have no injurious effect on the flowers.

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* The low yields of seed in this year were due to a very severe infection of mildew.

† It is proposed to experiment with powdered sulphur for this purpose next season.

Regular watering is of the greatest importance. To obviate the risk of drying, the pots are placed in saucers, but care is taken not to allow the soil to stagnate, as over-watering is almost as harmful as under-watering.

As a general rule about 400 to 500 florets of each cross are cross-pollinated, in most cases reciprocally. As emasculation is not necessary, the actual technique of cross-pollination is very simple, the material and appliances required for the work being some rather thin cardboard of the thickness of a visiting card for making the pollination cards (inferior quality tags will serve the purpose equally well), a pair of scissors, a bottle of alcohol, strong weather-proof tags with strong eyelets (it is convenient to have the tags in five or six different colours), a bundle of bast, and a number of small pollination bags 9 inches long and 6 inches wide, made of mineralised tiffany. The pollination is effected by means of small partly folded triangular pieces of cardboard about 2 inches long and about half-an-inch wide at the broad end, and tapering to a point at the other end. Two of these cards are used for each cross, one for tripping the flowers in readiness for pollination, and the other, the point of which is slightly roughened with the scissors, for collecting the pollen from ♂ parents and applying it to stigmas of the ♀ parents. Strong, vigorous heads on which about one-third to one-half the florets have opened are selected for pollination, but before starting the cross, the scissors and hands are sterilised and fresh cards prepared. The pollen of the ♀ florets, unless it is first removed, will after one or two pollinations cover the pollen of the ♂ plant to such an extent as to make it necessary to collect fresh pollen repeatedly. The first operation therefore is to remove the ♀ pollen by pressing the tips of the keels with the point of one of the cards. The ♂ pollen is scooped out with the point of the pollination card and placed on stigmas of ♀ florets by inserting the tip of the card between the standards and the keels and by applying slight pressure on the claws of the wings ; this causes the stigmas to be thrown forcibly against the pollen on the card. One collection of pollen will usually pollinate from 15 to 25 florets.

Various other kinds of pollinating instruments have been tried from time to time. The best results were obtained by using small camel hair brushes, but as these brushes are much more difficult to manipulate than cards, they are rarely used for pollinating red clover.

The pollinated heads of the plants which are to be placed in the open after crossing are tied together and enclosed in tiffany bags, which are attached to stakes. The bags are labelled with the temporary cross number and the numbers of the plants pollinated, and the date. After pollination the plants are kept in the glasshouses for four or five days—to allow sufficient time for fertilization to take place ; they are then placed in the open in shelters specially erected to afford protection from storms until they are harvested.

For most genetical crosses a somewhat different procedure is followed. In order to save time most of these crosses are made on young plants in the seedling year—an arrangement which fits in very well with the remainder of the work, as the seedlings do not usually start flowering before the beginning of September, while the pollinations for practical breeding are generally over by the end of August. When about 5 inches high some 4 to 10 seedlings of the families to be crossed are transplanted direct from the boxes in which the seeds were sown into large-sized pots and left in the open until they are nearly in flower. After they have been brought in they are staked in the usual way.

It will be shown later that when dealing with pedigree material it is generally necessary to inter-pollinate several plants within the same family. At first, sister plants were crossed in pairs in this manner :—1×2, 3×4, and 5×6, but when it was found six years ago that many of the intra-family crosses were completely sterile, the paired method was superseded for intra-family pollinations by the " chain " method, in which each plant is crossed with two other sister plants thus :—$1 \times 2 \times 3 \times 4 \times 5 \times 6 \times 1$. Using the same number of plants for both methods the chance of inter-pollinating cross-compatible plants is twice as great by the " chain " method as by the " pair " method.

Evidence has been obtained indicating that cross-incompatibility in red clover is determined by a series of multiple allelomorphs (Williams, 1931*a*). On this hypothesis if two plants ($S_1S_2 \times S_1S_3$) differing in only two factors for cross-sterility are reciprocally pollinated, and the F_1 plants from both parents inter-crossed at random, 37.5 per cent. are cross-incompatible : if, however, the F_1 plants of each ♀ parent are crossed *inter se*, as many as 50 per cent. are

cross-incompatible. In this case when the F_1 plants from the same ♀ parent are chain-crossed, it is possible from the results obtained to determine the fertility relationships of each plant with every other plant within the series crossed. An instance of this in which 10 F_1s from the same ♀ plant are involved is shown below:

Plant numbers ..	1	x	2	x	3	x	4	x	5	x	6	x	7	x	8	x	9	x	10	x	11	x	1
Assumed factorial constitution ..	S_1S_2		S_1S_3		S_1S_2		S_1S_2		S_1S_2		S_1S_3		S_1S_3		S_1S_3		S_1S_2		S_1S_3		S_1S_3		S_1S_2
Fertility relationship ..	+		+		O		O		+		O		O		+		+		O		+		

On the assumption that the plants in this family differed only in respect to two factors, or in other words, that the genetical constitutions of the two parents were S_1S_2 and S_1S_3, it is evident from the results of the chain-crossing that all the plants fall into two intra-sterile, inter-fertile groups, viz.: plants 1, 3, 4, 5, 9 with a factorial constitution of S_1S_2, and plants 2, 6, 7, 8, 10, 11 with a factorial constitution S_1S_3. On this hypothesis each plant is completely cross-incompatible with all the others within the same group, but is fully cross-compatible with every plant in the other group. Thus plant 1 is cross-sterile with plants 3, 4, 5, 9, but is cross-fertile with plants 2, 6, 7, 8, 10, 11. Though in this particular family all the possible combinations have not been tested, all the crosses that were made—some 19 in all—were in accordance with expectation. It is, therefore, clear in cases such as this that it is possible on the basis of the results from the preliminary chain-crosses to arrange the matings in such a way as to obtain seed from every cross—a very important consideration when dealing with valuable material. In the case of plants differing in four sterility allelomorphs, as for instance in families from $S_1S_2 \times S_3S_4$ parents, it is not always possible to predict with certainty their fertility relationships from the results of the chain-crossing, but as in these cases only 25 per cent. of the plants are on the average incompatible, this is not so necessary as in progenies with 50 per cent. incompatibles.

Reverting to the procedure employed in pollinating seedlings, as most of the crosses are made late in the season—September and October—the plants are kept under glass until the seeds are ripe. Though they are usually much healthier and produce better seed than old plants from the field they flower very irregularly, with the result that it often takes several weeks to complete one cross. Consequently, instead of bagging the crosses, as is done when the plants are placed in the open, the heads are labelled as they are pollinated, tags of the same colour being used for reciprocal crosses and tags of different colours for different crosses on the same plant.

Controlled cross-pollination by bees.

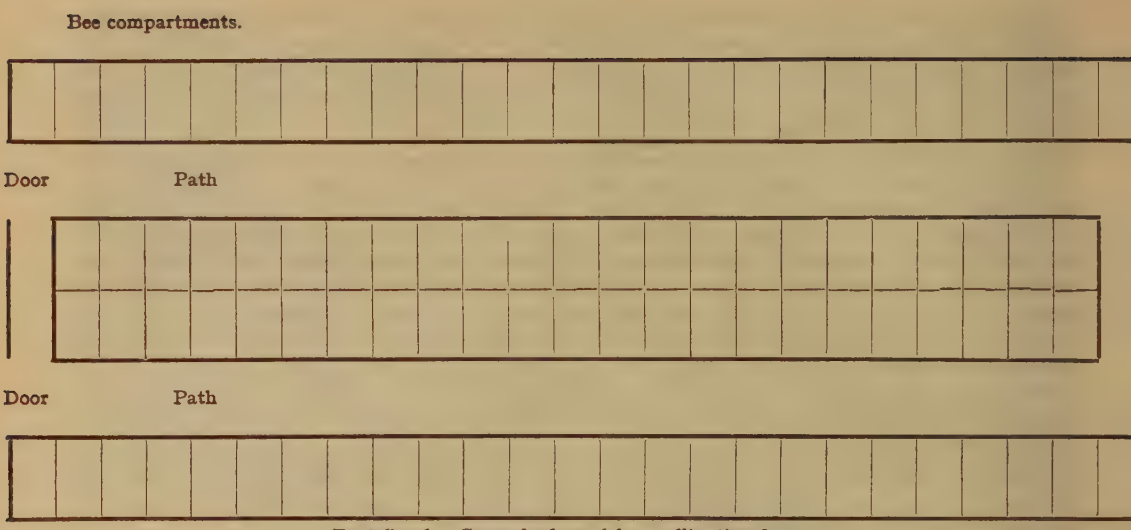
Since this method of crossing was instituted in 1921, the *modus operandi* has been greatly modified and improved. Before describing the technique now employed a brief account will be given of some of the methods formerly used.

Various types of cages for covering the plants to be cross-pollinated have been experimented with. During the first two years mosquito nets on wooden frames were tried with quite satisfactory results, except that they were exceedingly liable to be damaged by storms. Cylindrical wire gauze cages—3 feet in height and diameter—with the tops covered with muslin were then used, and though they were not entirely satisfactory, they gave much better results than some of the more elaborate cages used later. Of all the types tried, the least successful were the cages designed for the double purpose of pollinating cocksfoot and red clover. These consisted of strong wooden frames, 5 feet square and 5 feet high, with sloping glass roofs. In spite of the fact that for red clover work the linen side-panels used for cocksfoot breeding were replaced by muslin, the cages proved much too dark and damp for the purpose; the plants were frequently so badly drawn and mildewed that they produced no seed. For inter-crossing pairs of plants the type that gave the best results in the field was a small oblong cage, 3 feet in length and 2 feet 6 inches in width and height, made of strong wire gauze with 10 meshes to the linear inch fixed on a wooden frame, the top covered with a glass roof easily detachable for storing. Large cages of a similar type were used when several plants were being inter-crossed; for example, for crosses involving from 20 to 50 plants, cages 10 feet square were employed. (Plate II, figs. 6 and 7).

It is obvious that the selected plants must by some means or other be brought together so as to form a self-contained group for caging before crossing by bees can be carried out, as it seldom occurs that plants growing side by side are chosen. Various ways of bringing together the desired plants without unduly impairing their fertility have been tried. One method which was adopted with very indifferent results was to move the plants *in toto* from the field and replant them in their appropriate groups in a garden or convenient field. Even when the transplanting was done late in the spring, which is the best time for moving red clover, the casualties were usually very heavy, while the plants that were able to recover were often too weak to produce much seed. Another method tried was to cover *in situ* a few days before they came into flower the two plants to be inter-crossed, the stronger plant of each pair being protected by a pollination cage and the other by a small temporary cage. When the two plants were in full bloom, the one covered with the temporary cage was dug up and planted alongside the other in the pollination cage. Although the moved plant itself seldom produced any seed, it generally survived long enough to allow of its being inter-pollinated with the undisturbed plant. On the whole the method gave appreciably better results than that described above, in which both plants of each cross were moved, but it is much too laborious to be of general use. Several attempts have also been made to inter-cross the plants in such a way as to obviate the necessity of transplanting them. Both plants were caged and when they were in flower bees were transferred every 3 to 6 hours from one cage to the other, but as the bees were not amenable to working in double shifts, very little seed was obtained. Equally poor results were obtained by placing flower heads from one plant in the cage containing the other plant; for some reason the bees did not pay the slightest attention to the cut heads.

Although a considerable amount of time and thought had been devoted to the subject, very little headway was made in the technique of using bees for cross-pollination until 1924, when plants in pots were for the first time extensively used for the purpose. At first the plants were potted *in toto*, but in recent years cuttings have been exclusively used. To obviate the necessity of watering the plants while they were in the cages, the pots were sunk in the ground a few weeks before they were caged. During the dry warm summer of 1925 this method was an unqualified success, practically every cross producing an abundance of seed; even during the only moderately favourable season of 1924 the results were highly satisfactory. The summer of 1926, and especially that of 1927, on the other hand, were particularly unfavourable for bee pollination, the weather being cold and wet practically throughout the whole of the pollination seasons. Under these conditions the plants became very badly infected with mildew and anthracnose, and infested with green fly, while the bees were lethargic and perished in great numbers during the cold nights, with the result that a very large number of the crosses did not produce a single seed, while those which did produce some seeds were exceedingly poor. However, the costly set-backs received during the wet seasons of 1926 and 1927, when a large number of very promising plants died without having produced a single seed, served one useful purpose: they proved beyond doubt that no substantial progress in the improvement of herbage clovers was possible without ample glasshouse accommodation for controlled bee pollination, as well as for hand crossing. Consequently the following year a large glasshouse specially adapted for bee work was erected (Plate I, fig. 4). This house is 75 feet long, 20 feet wide, 4½ feet high to the eaves, and 12 feet to the ridge, and is designed in such a way that it can be readily partitioned off, in the manner shown in Text–fig. 1, by means of mineralised tiffany into 96 self-contained bee-proof compartments, each 3 yards square on the ground plan.

The two outside sections of compartments are generally reserved for crosses involving a fairly large number of plants, as for instance when a number of families are being combined together for the purpose of strain-building. In this case the cuttings of the selected plants of the families to be inter-crossed are planted in March in the bee compartments checker-board-wise, about 12 inches apart, direct from the small pots—the intervening partitions being removed if a cross occupies more than one compartment. Provided mildew and green fly are kept in check by judicious spraying and fumigation, most of the cuttings produce by midsummer strong, healthy plants with an abundance of flower heads; in fact, the growth under the glass is so rapid that it is often necessary to cut back the plants—particularly the early forms—about the middle of May in order to delay the flowering until an adequate supply of bees is available. Even then it is



Text-fig. 1. Ground plan of bee-pollination house.

necessary to stake the plants to prevent lodging. When the majority of the plants of a cross are in full flower, clean bees—one to each 6 to 8 plants—are introduced into the compartment and kept there for about three weeks before they are withdrawn. The seed yield obtained from the cross effected in this way is shown below, and since these figures speak for themselves it is hardly necessary to add that this method of crossing has proved very successful. One cross of 38 plants yielded over 50,000 seeds.

Year.	No. of crosses.	No. of plants.	Average no. of seeds per plant.
1929	17	275	509
1930	8	77	323

The two middle sections of the bee house are employed entirely for pair crosses. The plants used for this purpose are placed a few days before they start flowering in one of the ordinary greenhouses to protect them from promiscuous pollination by bees: they are then sprayed and fumigated. When in about the half-blown stage they are transferred, one pair to each compartment, into the bee house and clean bees are introduced—one for each pair. After a short period ranging from 4 to 7 days according to their activities, which in turn depend on weather conditions, the bees are withdrawn and cleaned in readiness for use for other crosses, and the plants are removed from the compartments to a large bee-proof cage and left there until they are harvested. This cage is 60 feet long, 12 feet high, and 12 feet wide, with sides of wire gauze; one half is roofed with glass and the other with wire gauze. By removing the plants to this cage as soon as they have been sufficiently cross-pollinated, it is often possible to use the same compartments for five or six crosses during a season. The average results obtained by this method during the last three years were :—

	Number of crosses.	Average number of seeds per cross.*
1928	28	1440
1929	36	725
1930	39	886

* These results refer only to out-crosses. The yields from in-crosses are complicated by the phenomenon of cross-incompatibility.

The bees are much more active in the bee house than in cages in the open, and they may often be seen busily at work in the bee house when the weather is too wet and cold for any to be out in the field. If ordinary care is taken, such as closing the house up at night and on cold days, the same bees will remain alive and active for three, four and even five weeks, provided the weather is fairly warm. If, however, the weather is very cold, the number of casualties may often be fairly heavy, but probably not heavier than under similar conditions in the open. In these circumstances it may often be necessary to obtain a fresh supply of bees, but under ordinary conditions one bee is generally sufficient to inter-pollinate one pair. The only bees now utilized for red clover pollination are workers of *Bombus hortorum* and *B. agrorum*, as experience has shown that these two species are better able to withstand handling and caging. Formerly they were caught on wild flowers such as wood sage and knapweed, or on red clover, but for the last two years a large bed of delphiniums has been used with great success as a decoy, as both species are apparently very partial to the flowers of this plant.

It is evident that bees captured on red clover flowers would have the pollen of this plant on their bodies, and it is equally evident as the writer has already shown (Williams, 1925), that if no steps were taken to clean the bees before they were used, some of the flowers of the plants to be inter-crossed would be pollinated with extraneous pollen ; and it does not require a profound knowledge of the principles of breeding to realize that for economic breeding and genetical study the value of such crosses would be greatly impaired, if not completely vitiated. Even when the bees are caught on flowers other than those of red clover, it does not necessarily follow that they are free from red clover pollen. Contrary to current opinion bees do not always confine their attention to one species during a flight, as both *B. hortorum* and *B. agrorum* have been observed on several occasions to visit three or four different species, including red clover, within the space of a few minutes.

The process of cleaning the bees is very simple ; it is based on the extreme readiness with which red clover pollen absorbs water—a fact first reported by Martin (1913). The pollen grains, which are normally elliptical in form, absorb water so readily that immediately they come in contact with it they swell up into spherical balls ; this causes a large proportion to burst, and in a comparatively short time they all become completely non-viable. The bees are trapped in large test-tubes in which they are also washed. The washing process consists in partly filling with water the tubes containing the bees and shaking them up for about half a minute ; the water is changed once or twice, care being taken that the bees are not kept immersed too long. The water is then poured out and the tubes are placed on a rack for two or three minutes until the pollen masses in the corbiculæ have dropped off ; the bees are again rinsed two or three times before they are placed to dry in small wooden boxes with wire gauze tops, made for the purpose. It is important that the boxes should be placed in a warm shady position, as the bees are apt to die very readily if exposed to direct sunlight while still wet. They are dry and ready for use in two or three hours after washing.

That this method is effective in cleansing bees of red clover pollen has been clearly demonstrated in several ways. Some of these tests have already been fully reported (Williams, 1925), and therefore only the results of some of the more recent experiments need be discussed here. In a number of cases the first flower head in each cross visited by the bees when introduced into the pollination cages was protected from further visits by enclosure in muslin bags. Since red clover is in the main self-sterile, the number of seeds produced by these heads would be a fairly good indication of the amount of foreign pollen still present on the bees after washing. The results obtained were as follows :—

Number of plants.	Approximate number of florets visited.	Total number of seeds.
14	760	3

The number of seeds actually obtained—0 .40 per cent.—is not incompatible with the number that would be expected if the plants had been self-pollinated by hand (see p. 47).

The effectiveness of the method was also tested by using washed bees for inter-crossing strains and pairs of plants without leaf-markings. Since in red clover absence of leaf markings

is a recessive character, it follows that the presence of plants with leaf markings in the F_1 progenies would be a sure indication that the washed bees used still carried some viable pollen. It is clear from the results below that the bees used in these tests were surprisingly free from viable pollen :—

Number of families.	Number of plants observed.	Number of plants with no leaf marking.	Number of plants with leaf marking.
8	3828	3827	1

HARVESTING AND THRESHING.

The rate of development of the seeds depends on weather conditions and the time of year when the pollinations were made. While the heads pollinated in June are usually ripe in about 30 days, and those crossed during July and August in about 35 days, the crosses made during September and October generally take 40 to 50 days to ripen. The general colour of the heads also affords a reliable indication of the state of ripeness of the seed, and all crosses in which most of the calyces are brown and the stems immediately below the heads are yellowish green are deemed to be ready for harvesting. In the case of the hand-crosses the bags are left intact, but in harvesting the bee-crosses the whole plant is cut and placed in a large paper bag. All the crosses are dried for several weeks in the glasshouses before they are stored.

The threshing is done during the winter by means of a small hand rubber. This consists of two open wooden boxes, 24 inches long, 12 inches wide, and 6 inches deep, one of which rests on the other. The upper box in which the threshing is done has strong wire gauze with 12 meshes to the linear inch across the bottom. The seeds are threshed out by rubbing the heads on the wire gauze, through which the seeds fall into the lower box, by means of a small appliance lined with corrugated rubber. This simple apparatus, besides being very effective for the purpose, has the additional advantage that it can be easily and thoroughly cleaned after each lot. With small lots the chaff is merely blown away, but for fair sized samples a miniature winnower is employed.

After threshing, the seeds of each cross are separated according to quality into two categories —good and fair, and counted, while the necessary data such as the number, quality, size and colour of the seeds are entered in the pollination books. Other routine work, such as the allocation of new family numbers, the preparation of history cards, the drawing up of sowing lists and the preparation of seedling books, has to be carried out before the sowing is done.

SOWING.

Before seeds are sown, the crosses are arranged according to the main object for which they are intended in their appropriate experiments, which retain the same numbers year after year. These experiment numbers are treated as self-contained units for most purposes, such as sowing, planting, and other field work, as well as for recording. But for a scheme of this kind, it would be impossible to deal efficiently with the large number of crosses involving scores of thousands of plants that are being studied every season.

The number of seeds of each cross sown depends on the number of plants required, or in other words on the relative importance of the cross ; for most purposes 200 to 300 seeds from each economic cross, and 400 to 500 seeds from each genetical cross are generally sufficient.

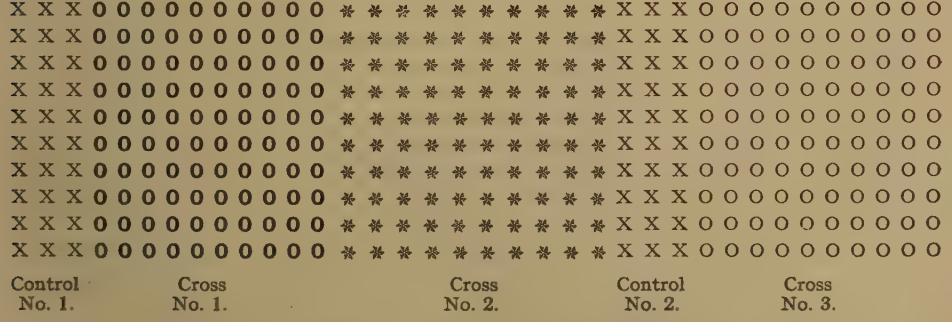
Most samples, despite the vigorous rubbing to which they are subjected in the hand thresher, contain a large proportion (in many cases 80 to 90 per cent.) of hard seeds, and unless these are treated before sowing so as to render the testæ pervious to water, they germinate very slowly and irregularly—many remaining dormant for a considerable time. The sulphuric acid method recommended by some workers is much too laborious for dealing with large numbers of samples. A very simple and equally effective method is to scratch the testæ by rubbing the seeds lightly between two sheets of sandpaper—the most intractable samples will give nearly 100 per cent. germination when treated in this way.

The seeds are sown in steam-sterilized soil in shallow wooden boxes about 20 inches long, 18 inches wide, and 3 inches deep. A little extra care in sowing the seeds at regular intervals in lines is more than compensated for by the time saved when counting and scoring the seedlings. The boxes are partly filled with soil which, after it has been levelled, is marked off in both directions into lines about one inch apart. The seeds are then dropped from a folded piece of cardboard on the points where the lines intersect, and covered to a depth of about half an inch with fine dry soil. This work, which to the breeder heralds the beginning of a new season, is usually started about the middle of January, and is often continued into April and even May. The genetical crosses are sown first in order to allow seedlings time to develop sufficiently to be studied during the ensuing summer ; the crosses for practical breeding are sown in March and early in April. During the winter months the seedlings are raised in heated houses, but from the beginning of April the seeds germinate equally well in cool greenhouses.

PLANTING.

All the crosses for practical breeding are planted out in the field generally in June, when the seedlings are about 6 inches high, but as regards the genetical crosses, it depends entirely on the nature of the characters under investigation whether they are studied in the boxes in which the seeds were sown or whether they are planted in pots or in the field. When studying the inheritance of certain chlorophyll characters, simple forms of leaf-markings, and other traits which can be easily determined during the early stages, the seedlings are scored in boxes, but when adult characters such as hairiness and colour of the flowers and seeds are to be investigated, the seedlings are planted in pots or in the field ; while all the crosses for the study of physiological characters, e.g., time of flowering, longevity, leaf size and vegetative vigour, are always planted in the field.

The method of planting the economic crosses is described below. Except that no controls are used, the scheme adopted for planting genetic crosses is very similar. In order to facilitate comparison, the crosses between the wild, late, and early plants are kept together in separate sections. Usually 100 to 150 seedlings of each family are put out in successive lines of 10 plants each, with controls of 30 plants every two families, in the manner illustrated in Text-fig. 2, and in Plate II, fig. 8. By arranging the seedlings in this way it is only necessary to label the families and control, as the individual plants can be easily numbered without the aid of labels. The distance between the lines is 27 inches, and between the plants in the lines, 20 inches. In order that there should be no marginal effect, there are no paths between the sections. Montgomery—as it is the most persistent variety in this district—is used as control for the wild and late crosses, and English broad red for the early crosses.



Text-fig. 2. Showing diagrammatically the arrangement of the breeding families and the controls in the field. The various signs indicate the positions of the plants.

After the ground to be planted has been finally rolled the whole area is marked into a series of parallel lines first in one direction and then at right angles. This is effected by hand drawn wooden markers made to draw 5 lines at a time. The seedlings are planted at the points where these lines intersect. For this work the most efficient unit is a party of 6 men, one man preparing the holes, another taking the seedlings from the boxes, the third placing them in the holes, and the other three doing the actual planting. Provided the seedlings are well developed and the work is done efficiently, the number of casualties due to planting is usually negligible. It is seldom necessary to water, as strong seedlings if well planted will stand two or three weeks drought without serious injury. In planting red clover, particular care has to be taken that the crowns of the seedlings are not covered, as the young shoots are unable to push their way through the soil; the writer has lost more seedlings through lack of attention to this point than from any other cause.

In addition to the spaced plants the economic crosses are planted out thickly—about 4 inches apart—in duplicated and triplicated lines of 40 plants each, chiefly with a view to studying their persistency and their general behaviour under competitive conditions.

FIELD OBSERVATIONS ON BREEDING MATERIAL.

All observations made in the field on pedigree material are entered directly into books specially designed for the purpose. As in the case of mixed populations planted for selection of parents, the only records taken in the seedling year on crosses for practical breeding, apart from some genetical observations on a few families that may be showing interesting segregations, are the mortality data and a few general notes on the families as a whole. Only in rare and extreme cases has any connection been observed between the amount of growth produced by the crosses in the first year and the performance of these crosses in the succeeding years. The only exceptions to this rule so far noted are families which produced in the first year (1) unusually luxuriant growth, and (2) very slow growth; the former were generally very short-lived, most of the plants failing to survive the first winter, while the latter, though often fairly persistent, were usually equally unproductive during the following years. In all the subsequent years careful observations are made three times each season—in spring, midsummer and early autumn—on each family and on every plant within the family. At each observation, marks are allocated to the individual plants as well as to the families as a whole indicating their relative agricultural values as estimated by eye; the family marks range from 1 to 10, and the plant marks from 1 to 5. The system of scoring the individual plants is shown on page 61 in a specimen copy of part of a page of a field note book.

A more accurate method of determining the values of the individual plants would be to weigh each one separately; though this is sometimes done in the case of a few important families, it is obviously impracticable when dealing with some thirty to forty thousand plants. Though the plant weights are not ascertained, the yields of each cross and each control as a whole are always determined in the second year, and sometimes in the third year, by cutting and weighing the hay and aftermath. These weights, when reduced to a common basis, such as yields per 100 plants alive in the first year, give a more accurate representation of the comparative values of the different crosses than do the family marks.

The F_1 families, even when they are derived from the same variety, often show very remarkable differences in regard to their yielding capacities (Plate II, figs. 9 and 11), persistency, winter-hardiness, disease resistance, and other important agricultural characteristics. As shown in the table on page 62, these differences are often quite apparent in the second year, but become more marked in each subsequent year. Families which give poor results in the second year have been found to fall off very rapidly in the next year, and it is therefore possible on this basis to discard many of the crosses at the end of the second year.

It does not necessarily follow that all the families which have proved highly productive in the second year will give equally satisfactory results in the third year. Even among the late × late crosses, in several instances families which have given very good results in the second year have disappeared almost completely by the spring of the third year. In some cases these non-persisting families died back from no apparent cause in the autumn, while in other families the plants were clearly winter-killed.

173(1)

Plant No.	1926	1927			1928			1929				Selected for crossing		Hairs.	Leaf marks.
	8/8 Casualties.	28/4	30/6	4/9	24/4	1/7	8/9	30/4	30/6	6/9		1928	1929		
1		3	4	3	4	3	2							A/S	Λ
2		2	4	3										S/S	Λ
3		5	5+	5	5	5	4	4	5	4		X	X	S/S	O
4		4	4	5	3	3	3							A/S	Λ
5		3	3	4	4	3	2							S/S	Λ
6		1	2	2	3	5	4	4	4	4				S/S	Λ
7	D														
8		5	4	5	4	4	5							S/S	O
9		4	4	4	3	4	4	1						S/S	Λ
10		5	5	5	5	5+	4	4	3	3		X		A/S	Λ
1		4	3	4	3	3	3	2	3	3				S/S	O
2		2	1											S/S	Λ
3		3	4	3	3	2	3	4	4	4				S/S	Λ
4		5	4	4	4	5	5					X		S/S	Λ
5		4	5	5										S/S	O
6		3	3	4	1	2	2	3	2	2				A/S	O
7		5	5	5	4	5	5	4	5	4		X	X	A/S	Λ
8		4	3	3	2	2	2	1	1	1				S/S	Λ
9		4	4	4	5	5	2	3	2	2				S/S	Λ
20		5	4	4	2	2	3							S/S	Λ
1		3	4	4	3	2	3	2	2	1				S/S	O
—		—	—	—	—	—	—	—	—	—				—	—
30		4	5	5	4	5	4	3	2	2				A/S	Λ

Specimen page of field note book.

Table showing the relative yielding capacities and persistency of some F_1 *families derived from Montgomery late. The yields are expressed in terms of lb. (green weight) per* 100 *plants alive in the early autumn of the first year and the persistency is expressed in the percentage number of survivors in May of each year—allowances being made for the casualties that have occurred as a result of planting.*

Year planted.	Family number.	Yield in 2nd year.	Percentage persistency		
			2nd year.	3rd year.	4th year.
1925	Montgomery controls	**84.5**	**92**	**52**	**22**
	156(1)	126.1	99	78	40
	156(1)	146.2	100	73	33
	168	112.0	97	76	44
	157(1)	65.6	100	93	57
	172(1)	87.7	96	66	15
1926	Montgomery controls	**78.2**	**94**	**52**	**8**
	230(1)	144.1	100	91	60
	228(1)	122.6	96	87	44
	232(1)	138.5	94	67	13
	238(1)	70.2	92	79	32
	227(1)	115.1	91	34	6
	233(1)	80.4	96	53	0
	237(1)	67.3	84	40	0
1927	Montgomery controls	**56.6**	**86**	**12***	—
	275(1)	95.1	97	23	—
	276(1)	96.3	88	14	—
	267(1)	73.8	100	36	—
	269(1)	41.9	86	43	—
	272(1)	45.6	89	11	—
	278(1)	24.1	97	0	—
1928	Montgomery controls		**54**	**25***	—
	350(1)	These crosses	52	42	—
	342(1)	were not	79	19	—
	344(1)	weighed.	17	5	—
	345(1)		28	0	—
1929	Montgomery controls	**62.8**	**95**	8	—
	485(1)	144.1	98	51	—
	483(1)	125.4	99	20	—
	503(1)	112.8	96	4	—
	480(1)	93.5	93	42	—
	482(1)	66.2	88	15	—
	344(1)	49.2	81	10	—
	484(1)	28.6	82	15	—
					—

* The winter 1928-9 was unusually severe, hence the low persistency results given in the third year by the 1927 plantings and in the second year by the 1928 plantings.

As would be expected from the extremely heterozygous nature of red clover, neither the performance nor the general appearance of the parent plants affords a reliable indication of the agronomic properties of their progenies, and experience has shown that the only trustworthy method of determining the true potentialities of the parent plants is by experimental breeding. This is clearly brought out by the results given below. In the first series of crosses shown, three late flowering plants were crossed with one and the same plant. Though the three plants did not appear to differ greatly in their yielding capacity, it is evident from a comparison of the results that their F_1 progenies show very wide variations in this respect ; the yield from 459(49) × 172(1)86 cross was about five times as much as from each of the other two crosses ; the plants of the first cross were not only far more persistent in the second year, but were also very much more productive individually.

F_1 progeny.

Parents crossed.	Second year.		Third year.
	Yield (hay) kilograms per 100 plants.*	Persistency per cent.	Persistency per cent.
Aa 459 (49) × 172 (1) 86	40.8	90.4	23.5
795 (1) 17 × „	8.8	30.7	3.3
796 (1) 16 × „	7.5	43.7	5.3

* Green weights (hay only) per 100 plants established in the seeding year.

In the second series of crosses, the plant Aa 459(49) which gave such outs andingly good results in the first series, as well as a number of F_1 plants of a family 201(1), were crossed with a few F_1 plants of another family, 200(1). If the yield and persistency results of these crosses are studied in conjunction with those given by the first series, it becomes evident that though the heredity complex of Aa 459(49) is such as to give rise to relatively short lived progenies, it possesses strong inherent potentialities for producing very heavy crops in the second year, a quality which is transmitted in a very marked degree to all its offspring :

Crosses.	Second year.		Third year.
	Lb. per 100 plants.*	Per cent. survivors.	Per cent. survivors.
459(1)49 × 200(1) 137	105.5	97.5	22.2
36	103.4	96.3	15.4
183	83.0	90.0	20.0
182	80.6	95.0	5.0
153	71.4	88.3	23.4
90	65.0	96.0	20.0
Average	**84.8**	**93.9**	**17.7**
201(1)89 × 200(1) 96	86.7	91.5	25.7
28 28	53.9	68.9	5.2
54 135	49.8	90.0	0
122 169	46.4	58.4	0
45 125	40.0	60.0	3.5
Average	**55.4**	**73.8**	**6.9**

* Green weights (hay only) per 100 plants established in the seeding year.

Data presented hereunder for two F_1 families obtained by crossing selected plants of Montgomery red also serve to illustrate the difficulty of predicting with any certainty the value of the progenies from the behaviour of the parents.

Family.	Second year results.	
	Yields in lb. per 100 plants.*	Per cent. survivors.
485(1)	165.0	98.1
484(1)	45.2	81.8

* Green weights (hay only) per 100 plants established in the seeding year.

The parents that gave rise to 484(1) family were considered to be rather better plants than those that produced 485(1) family, yet contrary to expectation the progeny of the latter proved far superior in productivity as well as in persistency to the progeny of the former.

These results do not imply that there is no advantage in making the initial selections; they merely serve to show that judgment on the value of the parents should be reserved until their progenies have been compared.

Although as shown in the table on page 62, there is not the least doubt that the progenies of carefully selected parents are on the average appreciably better than those from inferior plants, yet as a general rule only a comparatively small proportion—seldom more than 5 per cent.—of the F_1 families of even the selected plants are sufficiently outstanding to merit further attention. It is only the best plants of the most promising families that are selected for further breeding. In deciding on the best families, due consideration is, of course, given to the general performance of the families as shown by the family marks and the yield data during the whole period they have been under observation, while the plant marks form the basis on which the individual plants within these families are selected.

METHODS OF BREEDING.

The three methods of dealing with the selected F_1 material, namely, (1) strain-building, (2) brother × sister crossing, and (3) diallel crossing are described below. In the majority of cases all three methods are carried out simultaneously on the same material, and it is, therefore, often necessary for this purpose to take several cuttings from the same plants. (1) Strain-building: In adapting this method to F_1 material the main object is to meet the immediate demand for improved strains as expeditiously as possible. The probability is that many of these strains will be withdrawn later as better strains produced by slower but more effective methods are placed at the disposal of the farmer. In this method some 5 to 10 of the best plants from three to five of the most promising families that are fairly similar in regard to the main agronomic characteristics are inter-crossed by means of clean bees under glass in the manner described on p. 55. The same families are often used in different combinations. (2) Brother and sister crossing: The F_1 progeny from carefully matched pairs of plants often appear in a general way to be strikingly uniform. This uniformity is only apparent, and if the individual plants are closely examined they will generally be found to be very variable in a large number of characters such as plant size, persistence, leafiness, length and number of stems, rate of growth, hairiness, leaf size, and flower and seed colour, to mention only a few. The extremely heterozygous nature of the original parents will become still more evident when the F_1 plants are inter-crossed. Although the F_2 progenies often retain the general distinctive features of the F_1s, they almost invariably show a much wider range of segregation and, therefore, of variability than the latter; a large number of recessives such as various types of chlorophyll-deficient seedlings, lethals, dwarfs, leaf and floral malformations, which seldom appear in the F_1, segregate out extraordinarily freely in the F_2 generation. In the present state of our knowledge it is not possible to state definitely whether these recessive characters have any detrimental effect on the plants when present in the heterozygous condition, though it is not improbable, judging from the small amount of evidence at present available, that some of them have the effect of reducing longevity. But apart from these recessive characters, which may or may not prove harmful, most families contain during the early stages of breeding a number of undesirable types such as small, unproductive, stemmy and extremely hairy plants, and since red clover cannot be pure lined by self-fertilization, the only way by which these undesirable types can be eliminated is by a process of inbreeding in which brother and sister plants are inter-crossed.

When bees are used for inter-crossing, the sister plants selected for inbreeding are always crossed in pairs, but, for reasons already explained, when the operation is done by hand, from 5 to 10 plants of each selected family are usually chain-crossed. The F_2 plants from these crosses are planted in the field in the manner described for F_1s, and in due course plants from the best families are selected and inbred in the same way—this process being repeated for several generations unless the plants are homozygous for the most important characters. As a rule the plants become remarkably uniform after 4 or 5 generations of inbreeding.

This method of breeding is not nearly as simple as it appears on paper. As soon as he starts inbreeding red clover, the breeder is brought face to face with two serious problems, namely, loss of vegetative vigour and cross-incompatibility. The loss of vigour resulting from

inbreeding presents even greater difficulties in the improvement of red clover than cross incompatibility. The reduction is usually very pronounced in the second generation, becoming successively less at each subsequent crossing until the fourth or fifth generation is reached, after which there is no apparent loss. Though the rate and extent of the reduction vary within very wide limits in different families, the loss of vigour as measured by the productivity of the plants from F_1 to F_2 generations is of the order of about 40 per cent., and from F_1 to F_3 generations about 50—60 per cent. It should be stated, however, that a few families have been found which retain their vigour to a remarkable degree when inbred.

It is seldom necessary to inbreed for more than 4 generations, for by that stage the majority of the families are sufficiently homozygous for the purposes of practical breeding. Owing to the reduction of vigour, the inbred families as such are, for the most part, totally useless for practical purposes ; the vigour, however, can be immediately restored by out-crossing with unrelated plants. Therefore, to produce new strains from the inbred material, all that is necessary is to combine together the best families and the best plants in those families and to inter-cross them under controlled pollination conditions in the manner already described on p. 55. Fortunately for the plant breeder, inbreeding, except in so far as it decreases the chances of pollination between cross-incompatibles, has no direct effect on fertility or on the size and quality of the seeds.

(3) Diallel crossing. In this method one or two plants of the selected families are crossed diallely according to the scheme illustrated below, the object being to determine the combination of families or plants that will give the best progenies.

Families of male plants.	Families of mother plants.								
	163(1)	173(1)	228(1)	230(1)	247(1)	283(1)	297(1)	320(1)	348(1)
163(1)	—	+	+	—	+	+	+	+	+
173(1)			+	×	+	+	+	+	+
228(1)				×	+	×	×	+	+
230(1)					×	×	×	+	—
247(1)						×	+	+	+
283(1)							+	—	+
297(1)								+	+
320(1)									+

+ =crossed. × =crosses giving the best progeny.
— =not crossed.

In this case practically all the crosses are done by hand. Those families or plants which give the best combinations as measured by the performance of their progenies in the field are brought together and inter-crossed by clean bees to form a new strain. In the hypothetic series of crosses shown above, combinations involving the families 228(1), 230(1), 247(1), 283(1), 297(1) are shown as having on the average given the best progenies ; the new strain would, therefore, be built up entirely from these five families.

The inbred families are tested in like manner before they are finally selected and synthesized into new strains.

MULTIPLICATION OF PEDIGREE STRAINS.

The seeds obtained from the new synthetic strains are sown in boxes in the usual way, and 2,000 to 6,000 seedlings of each are planted out in well isolated areas in the counties for seed production. In choosing fields for this purpose the deciding factor is the remoteness of other red clover crops. The exact degree of isolation necessary to obviate contamination from cross-pollination with extraneous crops has not yet been determined, but the problem is under investigation ; the aim at present is to isolate the strains by growing them at a distance of at least half a mile from any other red clover.

The strains are carefully rogued early in June of the second year before the plants are in flower. The seed crops are harvested in the second year, and sometimes in the case of the most important strains in the third year as well. They are threshed by means of a small machine

specially designed for dealing with comparatively small lots of pedigree grasses and clover. This thresher is constructed in such a way that it can easily be cleaned after each crop, but as an additional precaution against the possibility of the strains becoming mixed, one or two lots of grasses are passed through the machine between each clover strain.

From these small islands which generally range in size from $\frac{1}{20}$ to $\frac{1}{4}$ of an acre, sufficient seed may be obtained for carrying out exhaustive trials as a preliminary to the multiplication of the best strains on a large scale.

RECORDS.

Allocation of sample numbers.

To facilitate recording, all the most important crops under investigation at this Station are represented by symbols. The four main groups into which these crops are divided, namely, Leguminosæ, Gramineæ other than Cereals, Cereals, and " Other Crops," are represented by capital letters, A, B, C and D, respectively, while the species within these groups are indicated by small letters ; thus the Station symbol for red clover is Aa, for alsike Ab, for white clover Ac, for lucerne Af, for *Lotus* spp. Al, and so on.

Starting from unity for each species, station numbers are allocated to each lot of plants or sample of seed immediately it is received. These numbers, together with the necessary particulars concerning the samples, are entered in the appropriate sample ledger, there being one for each of the four groups referred to above. In addition, history cards are drawn up for recording the history of each sample, the number of the experiment in which it is sown, and other relevant information.

Recording of field data.

The work of collecting and collating the data is greatly simplified by using different note-books for recording data during the different phases of the breeding operation. Particulars of manual operations, except cutting and weighing, such as cultivation, manuring, planting, propagating and potting, are recorded in an " operation book ". It has been found convenient to keep all yield data together in a " weighing book ". The procedure in this case is to record the weights of each plot direct on to a ground plan of the experiment. As previously stated, red clover segregates very freely for a great many interesting seedling characters. These segregations, together with the data relative to sowing and germination, are scored in specially prepared " seedling books "—one page being allocated to each cross for the purpose. Full particulars of each family—the object of the cross, lists of the characters to be studied, and description of the parents concerned—are shown in these books ; these particulars are transferred to the " field note-books " when the seedlings are planted in pots or in the field.

The field note-books used for recording field data in connection with practical breeding have already been described ; similar books are employed for scoring the genetical material in pots as well as in the field.

Mention has previously been made of two other books—namely, the " study book " in which detailed observations on the plants to be crossed are entered, and the " pollination book".

Starting from unity each year every cross is given a temporary number. It is an excellent scheme to allocate to each species to be crossed a block of cross numbers, and then to assign to related crosses within a crop a series of consecutive numbers. The arrangement shown below illustrates in a general way the scheme usually adopted; the exact number of crosses allocated for different purposes will naturally vary from year to year. To facilitate recording, the crosses are arranged as far as possible in the order in which they are to be sown and planted in the following year.

Sub-groups.	*Divisions.*	*Temporary cross numbers.*
Economic crosses (Bees) 1 to 200 ..	Strain-building	1—30
	Wilds	31—50
	Lates	51—150
	Earlies	151—200
Economic crosses (Hand) 201—400 ..	Wilds	201—240
	Lates	241—350
	Earlies	351—400
Genetical crosses (Hand) 401—650 ..	Chlorophyll deficients ..	401—430
	Flower and seed colour ..	431—500
	Hairiness	501—540
	Leaf markings	541—590
	Dwarfness	591—620
	Earliness	621—630
	Vegetative vigour ..	631—650

The particulars entered in the pollination books are : temporary cross numbers, method of crossing, numbers of the plants crossed, objects of the cross, brief notes on the characters of the parents to be crossed, number of heads and florets pollinated, date of pollination and harvesting, number of bags used, and notes on the condition of the flowers. The number, colour and quality of the seeds, and new family number are entered during the winter after the cross has been threshed. A specimen copy of a page from a pollination book is given below.

X 534 $632_{(1)}$ 1 × 2

Pollinations.

Hand		Plt.	Date.	Bags.	Hds.	Florets.	Remarks	Initials.
	Flr. col. Rose P × ditto P26, 67'C.	(1)	6/8	—	1	57	Pollen abundant.	L.M.
Genetics.	Anthocyanin + + × + +		12/8	—	3	141		L.M.
	L. mkgs. ∧ w. c. med. × O					198		L.M.
	Hairs $\frac{A + A + A + S}{P}$ × ditto $\frac{}{O}$	(2)	12/8	—	4	223	Pollen abundant.	

New number.

$632_{(2)}$ 1 × 2

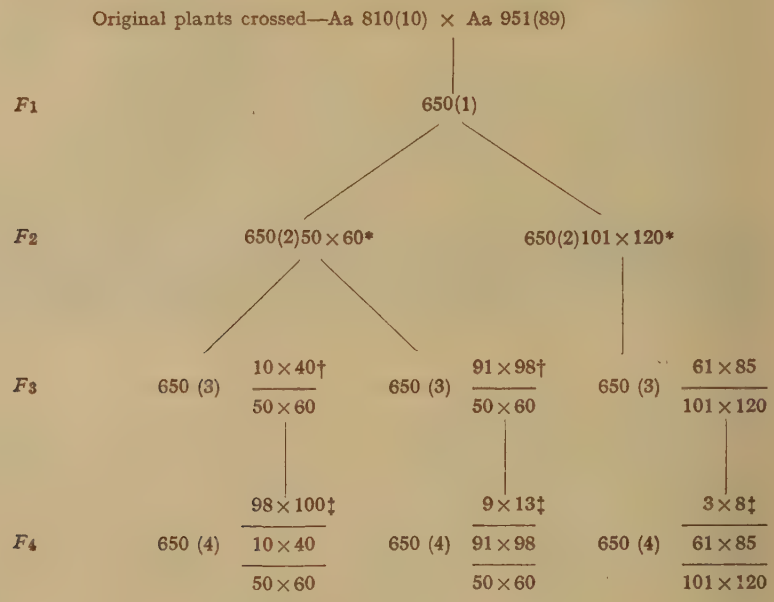
Seeds.

Plt.	Harvested.	Gd.	Number Fair.	Total.	%	Col.	Size.	Quality.
(1)	15/9	129	6	135	66.6	P_1C_8	Large	V. gd.
(2)	18/9	142	4	146	65.6	$P_{10}C_{10}$	,,	V. gd.

Hybridization numbers.

The F_1 seeds from all original crosses, that is, crosses other than those between brother and sister plants, are given new family numbers which should not be confused with the sample numbers. Starting from unity for each species the family numbers together with a brief description of the parents and other particulars are entered in a " Hybridization ledger". The family numerals are distinguished from sample numbers by reversing the symbols, thus Aa is

changed to aA, and Ab to aB, and so on, but as a rule they are seldom used except for permanent records. The progenies resulting from cross-fertilization are differentiated from those arising from self-fertilization by placing the symbols behind the numerals. Crosses between plants within the same family and in the same generation always retain the original family numbers, but all back- and inter-family crosses as well as sub-families resulting from inter-crossing plants of different generations of the same family are allocated new family numbers. The different generations are indicated by bracketed numerals, and these are followed by the numbers of the plants crossed. A brief outline of the system of numbering employed is shown below.



* The numbers of the two F_1 plants crossed to give the F_2 sub-family.
† The numbers of the two F_2 plants crossed to give the F_3 sub-family.
‡ The numbers of the two F_3 plants crossed to give the F_4 sub-family.
The plant numbers start from 1 in each family and sub-family.

This method of numbering is very simple ; it has an additional advantage in that it shows at a glance the exact lineage of each sub-family. For the purpose of labelling and field work in general, the plant numerals with the exception of those of the last pair crossed are generally omitted.

History Cards.

The history and description of each plant crossed are briefly recorded on cards on which are also shown the number of the experiment concerned and the numbers of the books in which more complete data may be found. Each original cross has a main guide card ; the other cards are arranged in sequence according to the generation behind these main cards, distinctive coloured cards being used for the different generations. The specimen copies shown below will serve to illustrate the kind of information entered on the cards.

630(1)

1928
× 480

Aa 1532 × 188 (2) $\frac{22}{9 \times 27}$

Wild × B.R.

Genetics.

Flower colour.

Seed colour.

Anthocyanin.

Leaf marking.

Hairiness.

Main guide card. 6″ × 4″.

P

1929 (× 480) 630(1) 188 (2) $\frac{\text{Aa 1532}}{\text{22}} \times \frac{}{9\times27}$

History.—Aa 1532 wild red selected from an old meadow, at.....Montgomeryshire, 1927.

188 (2) $\frac{22}{9\times27}$ B.R. ex A. 98 (1927) B. 178.

Description. See Study book 313.

	Aa 1532.	188 (2) $\frac{22}{9\times27}$
Flr. col.	M1	W (pure)
Anthocyanin	+	O
Seed col.	P10+	Ivory white
Leaf mkgs.	Basal $\frac{2}{3}$ area	O
Hairs	$\frac{A+O+O+O}{P/O}$	$\frac{A+Atr.+Str.+O}{P/+}$

Parent card. 6″ × 4″.

F_1

$630_{(1)}2$

History : *ex* A 98 (1929) B. 370.

Description. See Study book 313.

Flr. col. d. rose P. Ridgeways P26, 67' d.

Anthocyanin +

Seed col. P_2

L. mkgs. ∧ W. medium.

Hairs $\frac{A+A+A+Str.}{\frac{P}{O}}$

Crosses 1930 2×1 = × 520 Polln. B 390
2×3 = × 521 „ „ „

2×188(2) $\frac{22}{9\times27}$ =841(1) (×526) Polln. B. 390

F_1 card. 6″ × 4″.

Index cards.

In addition to the history cards, index cards giving a complete list of all the plants crossed and which have produced seeds are also kept for each original family number. One such card is illustrated below.

$200_{(1)}$

Year.	Parents.	Books.	Year.	F_1	Books.	Year.	F_2	Books.
1925	459 (49) × 502(50) } 200(1)	161	1927	1×2	211	1928	1×2 (6)×459(49)=620(1)	240
				2×3	,,		1×2(40)× ,, =621(1)	,,
				4×5	,,		4×5(89)×344(1)60=642(1)	,,
				1×5	,,			
				2×459(49)=401(1)	212			
				4×459(49)=402(1)	,,			
1925	459(49) × 485(101) } 194(1)	159						
			1928	50×51	238			
				51×52	,,			
				101×672(81)=580(1)				

Family index card. 6″ × 4″.

WHITE CLOVER.

As in the case of red clover, white clover is mainly, though not entirely, cross-fertile. The chief pollinating agents are honey bees and short-tongued humble bees such as *Bombus terrestris*. On reference to the data presented below it will be seen that it is to a very slight extent autogamous in the absence of pollinating insects.

The number of seeds obtained from white clover plants as a result of spontaneous self-pollination was as follows :—

Year.	No. of plants isolated.	No. of plants producing seeds.	No. of flower heads.	Total no. of seeds.	Aver. no. of seeds per head isolated.
1927	50	7	349	44	0.133
1928	80	10	1406	90	0.064

The data recorded below show the average number of seeds obtained from 11,620 florets which were artificially self-pollinated.

Year.	No. of plants involved.	No. of plants that produced seeds.	Total no. of florets artificially selfed.	Total no. of seeds.	Seeds per cent. florets selfed.
1928	62	16	7329	153	2.1
1929	40	11	4291	230	5.4

If these figures are compared with self-pollination results obtained for red clover (see p. 47) it will be evident that white clover is appreciably more self-fertile than red clover. For instance, in the case of red clover, the average number of seeds per 100 selfed florets obtained in 1928 and 1929 was 0 .69 and 1 .14 respectively, while the average percentage self-fertility of white clover in the same two seasons was 2 .1 and 5 .4 respectively. The different plants show wide variation as regards the degree of self-fertility. Most of the plants appear to be completely self-incompatible. Some are very slightly compatible, while an occasional plant—probably not more than 1 to 2 per cent. of any population—may be found exhibiting a relatively high degree of self-fertility. For instance, of the 102 plants mentioned above, only 27 produced any seeds on being artificially selfed, and of these, 20 plants yielded from 1 .5 to 10 per cent. seed, 6 plants from 10 to 26 per cent., and 1 plant as many as 102. 5 per cent.

By virtue of the fact that it is rather more self-compatible than red clover there are greater possibilities in white clover of isolating self-fertile lines, but owing to the high degree of self-sterility of the crop as a whole, and the pronounced loss of vigour which occurs as a result of inbreeding, the chances of producing improved strains even of white clover by repeated self-fertilization are very small. A method of breeding somewhat similar to that adopted for the improvement of red clover offers, in the opinion of the writer, much better prospects of success.

During the initial stages of breeding the plants are crossed by hand either in pairs, chainwise, or diallely. The pollination is done under glass in the way described for red clover, that is, by means of pollination cards. Since white clover produces from 2 to 5 seeds per pod as compared with 1 and very occasionally 2 in red clover, sufficient seed is generally obtained for most purposes by pollinating 200 to 250 florets on each pair. The pollinated heads are seldom covered—the peduncles being generally too short, slender and weak to allow of bagging. To protect the flowers from bees, the plants are kept in the pollination houses until the seed is harvested. Curiously enough, white clover is seldom attacked by mildew or troubled by green fly.

The average numbers of seeds from crosses between unrelated plants for 4 seasons are given below.

Year.	No. of crosses.	Seed per cent. florets crossed.
1927..	42	75.0
1928..	53	100.4
1929..	17	121.3
1930..	41	104.8

As in the case of red clover, when sister plants within one and the same family are inter-pollinated, some of the crosses are fully compatible, while others are completely or almost completely reciprocally incompatible, and there appears to be no doubt from the evidence already obtained that cross-sterility in white clover, as in red clover, is governed by a series of multiple allelomorphs (Williams, 1931*b*). Of the 137 $F_1 \times F_1$ crosses made during the last three seasons 37 were incompatible. There was no significant difference between the fertility of compatible sister × sister crosses and that of the crosses between unrelated plants.

It is essential if observations are to be made on the rate of spreading of the individual plants, which is obviously one of the most important characteristics of white clover, that the seedlings should be planted out at least 3 feet, or preferably 4 feet, apart in all directions. To prevent self-seeding with its resultant contamination, the flower heads should be hand picked periodically during the summer.

White clover is an extremely variable species, and contains an extraordinarily wide range of types differing greatly in their agricultural values (Plate II, fig. 10). This vast store is as yet untapped by the plant breeder, but there is not the least doubt that greatly improved forms can be produced by systematic breeding. The results given below are interesting, as they serve to indicate the extreme variability that exists even within indigenous wild white clover as well as the possibilities of effecting improvement by breeding. All the families involved were obtained by crossing in 1927 indigenous wilds in pairs. The F_1 progenies were planted out as spaced plants—60 to 120 of each family—in the spring of 1928. As it is not possible to obtain accurate yield data on spaced plants of white clover, the relative productivity of the different families is indicated by marks ranging from 1—10.

	Second year.		Third year.	
Family number.	Per cent. survivors.	Relative productivity.	Per cent. survivors.	Relative productivity.
55(1)	92	9	91	10
56(1)	94	7	93	10
60(1)	82	9	82	8
91(1)	96	7	90	9
58(1)	92	4	87	6
68(1)	77	2	70	2
57(1)	98	9	87	2
81(1)	92	10	75	2

It will be observed that families 57(1) and 81(1) which were outstandingly good in the second year proved almost complete failures by the third year. In marked contrast to these two short-lived families and to family 68(1), a very slow growing type, are 55(1) and 56(1), which produced better swards in the third than in the second year.

To produce new strains the best plants of the most promising families are combined together as described for red clover. The crossing is done by clean bees—*B. terrestris* being used for the purpose, as they have been found to stand handling much better than honey bees. Instead of planting the cuttings direct in the bee-pollination house as is done in the case of red clover, the plants are pollinated in pots. It has been noted that they flower more profusely and set seed more freely under conditions restricting vegetative growth than when they have unlimited room for spreading.

LUCERNE.

Although the breeding of lucerne has been in progress at Aberystwyth since 1925, it has not been possible to devote as much attention to this work as has been paid to the improvement of red and white clovers. Therefore, a fair amount of experimental work still remains to be done before it will be possible to state definitely whether the general scheme outlined below is practical in all its parts. The indications are that lucerne is amenable to a more direct line of attack than is possible in the case of the clovers.

Though the natural mode of reproduction of lucerne is by cross-fertilization, it has been independently demonstrated by several workers that the crop as a whole is capable of effecting self-fertilization to quite a considerable extent. A small proportion of plants have been found to be more or less completely self-incompatible, but most of the plants produce a few seeds in the absence of pollinating agents, and appreciable, though varying, amounts when artificially self-pollinated. In the breeding of lucerne advantage is taken of this fact. The method adopted is as follows : in 1925 some 96 plants selected from a number of varieties of proved merit were self-pollinated ; in the following year the progenies of these plants were planted in the field, 3 feet apart, and kept under observation for two or three years, when the best plants in the most outstanding lines were again selfed. L_2 progenies were again planted out in a similar manner for study and selection. As lucerne is an open pollinated crop all the original plants selected naturally proved more or less heterozygous for a number of important characters, though it must be stated that the range of segregation within each line in the L_1 generation was far less than was expected, and in the L_2 generation many of the lines were remarkably uniform. Inbreeding, however, is almost invariably accompanied by a very rapid fall in vegetative vigour, the reduction being very marked from the L_1 to the L_2 generation. Some of these L_2 lines have now been selected for further inbreeding, which will be continued for 1 or 2 more generations, but other lines which seem to be moderately homozygous as regards their main agronomic characteristics have been selected for crossing. These will be crossed during the coming season in a diallel manner, the intention being to ascertain the combination of lines that will give the best progenies with a view to combining these into new strains later.

The self-pollination of lucerne is a very simple operation, as the flowers are easily tripped by pressing them gently between the fingers, and in most cases this can be readily effected by drawing the racemes through the hand. In crossing, it is hardly necessary for the purpose of practical breeding to emasculate the flowers—a difficult and tedious operation in the case of lucerne. The method usually employed is to insert a stout pin horizontally between the standard and the keel in such a way as to prevent the stigmas from coming in contact with the standard when the flowers are tripped. Any pollen that may be on the stigmas is brushed off before the pollen from the male parent is applied.

During the first few years the pollinations were carried out entirely *in situ* in the field, bee-proof wire gauze cages being usually employed for protecting the plants, which were selfed every 7 to 10 days throughout the flowering period. As may be seen from the data presented below, the results obtained in 1925, which was a moderately favourable year for seed setting, were fairly satisfactory, but during the inclement summer of 1927 very little seed was secured.

Season.	Generation of plants selfed.	No. of plants selfed.	No. of seed-producing plants.	Average no. of seeds per plant for all the plants selfed.
1925 Field	Parents	96	73	246
1927 Field	Parents	22	10	7
	L_1	63	19	6
1928 Greenhouse.. ..	Parents	39	39	1541
	L_1	73	61	329

After the disastrous experience of 1927 all the pollinations have been effected on cuttings in glasshouses, a method which has proved eminently successful, as the data for 1928 indicate.

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——————— (1931*b*). "Self- and cross-sterility in white clover." *Welsh Plant Breeding Station Bulletin*, Series H., No. 12.

EXPLANATION OF PLATES I—III.

PLATE I.

Fig. 1. Inside view of cocksfoot isolation house.

Fig. 2. Inside view of grass hybridization house.
Note (*a*) manner in which the envelopes are turned over and clipped at the corners ; (*b*) method of attachment of the top of the envelopes to the supporting stakes ; (*c*) very large wad of cotton wool around the base of the inflorescences which permits of a strong tie to the stake.

Fig. 3. Self-pollination of cocksfoot in the field.
Note (*a*) the stout dahlia stakes as supports ; (*b*) the frames over which the " balloons " are stretched ; (*c*) the thick wads of cotton wool at the top and bottom of each " balloon " which permit of strong ties.

Fig. 4. Inside view of house used for controlled pollination with bees.

PLATE II.

Fig. 5. Photograph taken early in the summer of some red clover plants grown from cuttings taken during the previous autumn.

Fig. 6. Small wire gauze pollination cage with two tiffany pollination cages on the right.

Fig. 7. Different types of pollination cages. A cylindrical cage in the immediate foreground ; a small rectangular wire gauze cage and a large tiffany cage in the background.

Fig. 8. Arrangement of pedigree families in the field.

Fig. 9. A very persistent family of Montgomery red clover in the third year (in the background) with ordinary Montgomery red clover controls (in the foreground).

Fig. 10. Two bred strains of white clover. Left foreground, a moderately high yielding family ; right foreground, a low yielding family.

Fig. 11. In the foreground two F_1 progenies from pairs of selected plants of Montgomery red clover in the second year. Left : $504_{(1)}$ a highly productive family. Right : $344_{(1)}$ a comparatively low yielding family.

PLATE III.

Cotton fabrics used in experiment B. 183. (See p. 11). × 12. (Photographs by the Shirley Institute, Didsbury, Manchester.)

PLATE I.

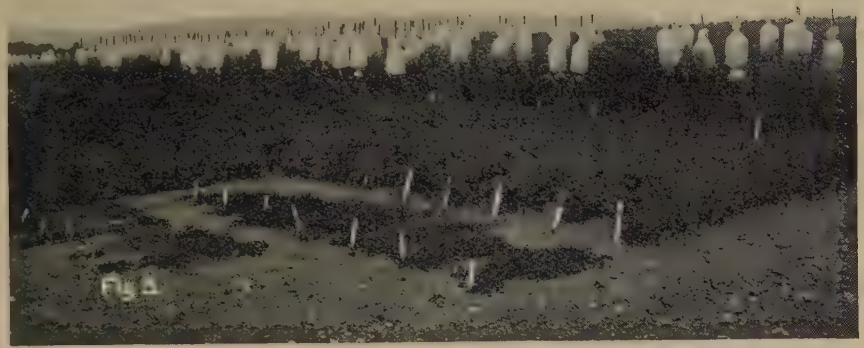
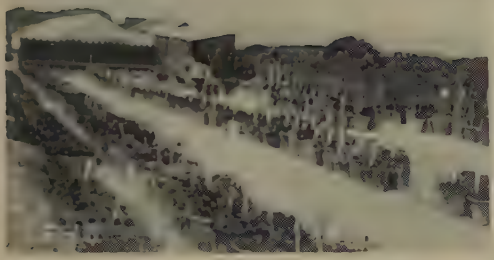


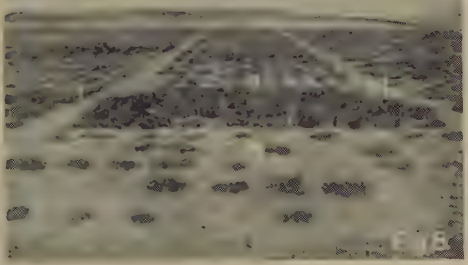
Fig. 3.

Fig. 4.

PLATE I.

PLATE. II.





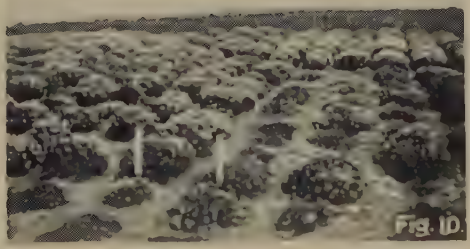


Fig. 10.

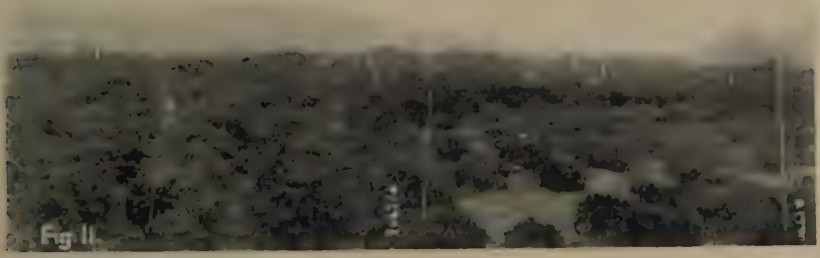
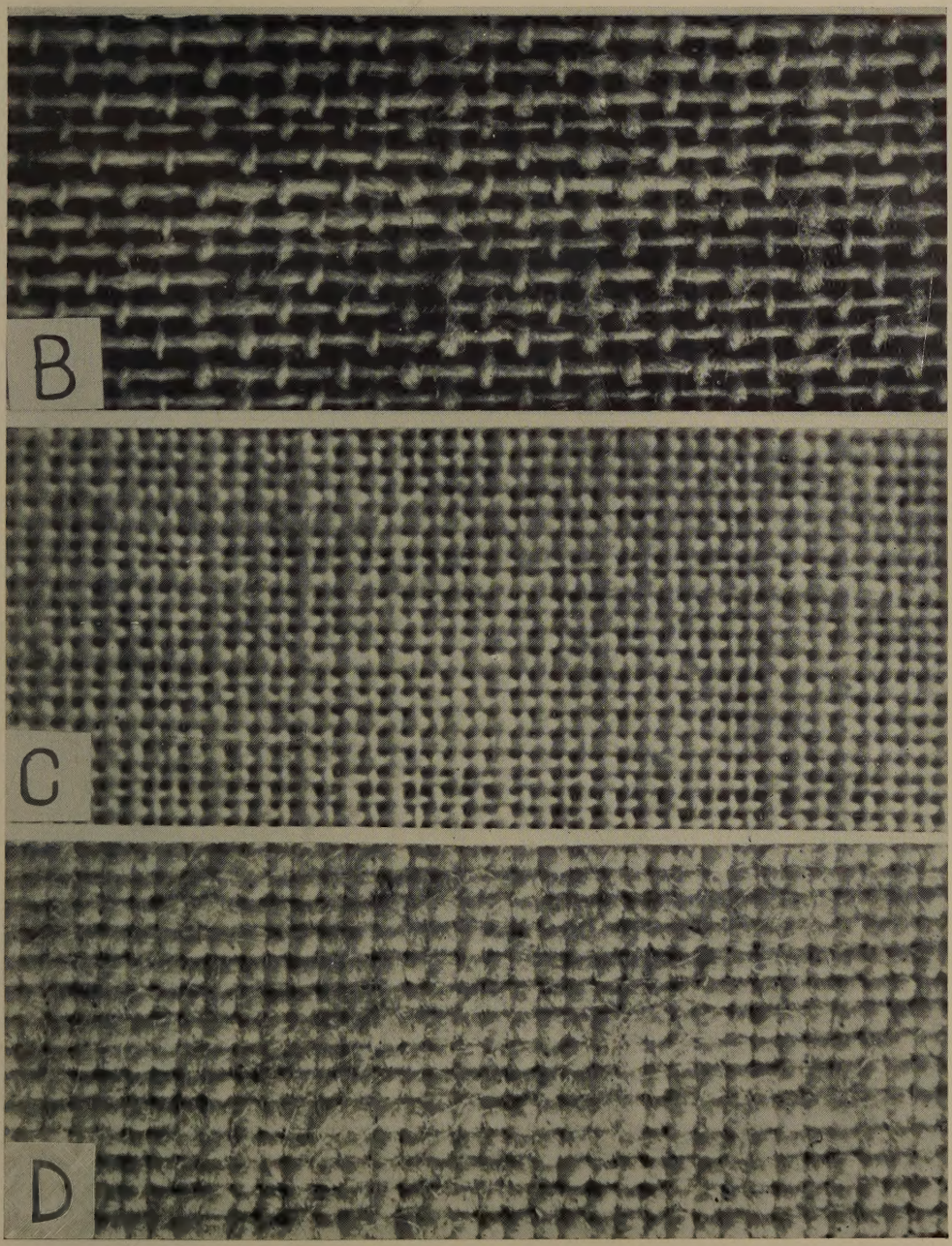


Fig. 11.


B
C
D

PUBLICATIONS OF IMPERIAL BUREAU OF PLANT GENETICS: HERBAGE PLANTS

BULLETIN SERIES (Irregular).

Bull. No. 1. Miscellaneous information relating to Breeding of Herbage Plants. March, 1930. (1s. 6d.).

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HERBAGE ABSTRACTS. A quarterly issue, commencing June, 1931, superseding Current Literature Lists, Herbage Research Circulars and Monthly Abstract Service. [1s. 6d. per copy or 4s. for 1931 set (3 issues only). Thereafter 5s. per annum.]

The Library of the Welsh Plant Breeding Station and Imperial Bureau of Plant Genetics, Herbage Plants, is anxious to complete its sets of certain periodicals, and would therefore appreciate information leading to the acquisition of the parts or volumes enumerated below:

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